Modular regulation analysis of integrative effects of hypoxia on the energetics of contracting skeletal muscle in vivo

Christophe BEUSTE, Sylvain MIRAUX, Véronique J. DESCHODT-ARSAC, Eric THIAUDIERE, Jean-Michel FRANCONI, Philippe DIOLEZ and Laurent M. ARSAC

Résonance Magnétique des Systèmes Biologiques, UMR 5536 CNRS (Centre National de la Recherche Scientifique) – Université Bordeaux 2, Bordeaux, France

In the exercising muscle, acute reduction in ambient oxygen impairs muscle contraction because of the effects of hypoxia on mitochondrial ATP supply. The less marked impairment reported after long-term exposure to hypoxia points to changes in the regulation of the energetic system of contraction in HC (hypoxic conditioned) animals. This energetic system is conceptually defined here as two modules: the ATP/PCr (phosphocreatine)-producer and the ATP/PCr-consumer connected by energetic intermediates. Modular control analysis that combines top-down control analysis with non-invasive 31P-NMR spectroscopy was used to describe the effects of hypoxia on each module and their adaptation. Modulations of steady levels of ATP turnover (indirectly assessed as force output) and muscle PCr were obtained in HC rats (6 weeks at 10.5% \( \text{O}_2 \)) compared with N (normoxic) rats. Modular control and regulation analyses quantified the elasticity to PCr of each module in N and HC rats as well as the direct effect of acute hypoxia on the ATP/PCr-producer module. Similar elasticities in N and HC rats indicate the absence of response to long-term hypoxia in internal regulations of the ATP supply and demand pathways. The less marked impairment of contraction by acute hypoxia in HC rats (−9 ± 6\% versus −17 ± 14\% in N rats, \( P < 0.05 \)) was therefore fully explained by a lower direct effect (HC −31 ± 13\% versus N −44 ± 23\%, \( P < 0.05 \)) of acute hypoxia on mitochondrial ATP supply. This points to a positive adaptation to chronic hypoxia. Modular control analysis in vivo may provide powerful tools to find out improved function (alternatively dysfunction) at the system level in conditioned animals.

Key words: ATP turnover, hypoxic conditioning, metabolic control analysis, oxidative phosphorylation, 31P-MR (magnetic resonance) spectroscopy, skeletal muscle.

INTRODUCTION

As an effective oxygen sink, mitochondria house the ultimate step of the fate of the oxygen pathway from air through the cardiovascular and pulmonary systems. Oxygen is used for substrate oxidation by mitochondria and is therefore involved in the main mechanism responsible for energy supply in moderately exercising skeletal muscle. An intriguing question for physiologists over the last three decades has been the influence of acute hypoxia on muscle contraction in an integrated context. Accordingly, the effect of inhaling gas mixtures with varied fractions of inspired oxygen on muscle respiration has been extensively studied. Acute and severe reduction in oxygen delivery typically results in a wide range of metabolic disturbance, e.g. decrease in the steady level of PCr (phosphocreatine) [1] and alteration in other energetic intermediates (ATP, P, and ADP) [2], ultimately culminating in a total loss of contractile function [3,4]. A less marked perturbation by acute hypoxia of the bioenergetic system of contraction in skeletal muscle is generally reported in humans as well as in rodents after exposure to hypoxia for several weeks as well as in humans indigenous to high altitudes [5,6]. To date, the hypoxia-induced responses of integrative regulatory features of muscle energetics have not been systematically studied.

Recent successful analyses suggest that an improved understanding of the integrative regulation by \( \text{O}_2 \) could be gained from a modular approach of the energy metabolism of contracting muscle. In such integrative approaches, the energy metabolism of intact contracting muscle (either heart or skeletal muscle) can be conceptually divided into two modules: the ATP/PCr-producer and the ATP/PCr-consumer [7–10]. Modules communicate only via a group of energetic intermediates (ADP, ATP, P, creatine and PCr) whose concentrations are connected with each other and with the phosphorylation potential. Since muscle PCr dynamics can be detected non-invasively by 31P-MR (magnetic resonance) spectroscopy with a high signal/noise ratio, [PCr] (PCr concentration) is systematically the studied variable in the above experimental works. The reactivity of each module to PCr is quantified by the elasticity coefficient (or elasticity). Since the complete set of elasticities determine internal regulation in a metabolic pathway [11–15] as well as in modular systems [7–10,14,16,17], these parameters may be used to analyse the regulation of ATP turnover or the so-called 'energy demand-supply coupling' [2,5,6,18] in contracting muscle of hypoxic animals. Additionally, once modular elasticities are known, the regulatory role of any effector on the energetic system can be quantified [9,12]. So, the overall regulation analysis would likely make it possible to find out the origin of the less marked perturbation by \( \text{O}_2 \) of the energetic system of contraction after hypoxic conditioning.

In the present study, 31P-MR spectroscopy was used to quantify in vivo the elasticity to PCr of the ATP/PCr-producer and ATP/PCr-consumer modules in N (normoxic) rats compared with HC (hypoxic conditioned) rats exposed for 6 weeks to 10.5% \( \text{O}_2 \). The determination of elasticity values then served for the determination of control exerted by each module on ATP turnover.
and contraction, as quantified by flux-control coefficients. At last, thanks to the determination of control distribution, the direct effect of \(O_2\) on the producer module was quantified in N and HC rats to uncover regulatory features of acute hypoxia on the energetic system.

**EXPERIMENTAL**

**Animal preparation**

Female Wistar rats weighing 280–340 g were used for these experiments according to the guidelines of the National Research Council’s Guide for the Care and Use of Laboratory Animals. All animals were housed four to a cage in an environmentally controlled facility (12 h light/12 h dark cycles, 22 °C) and received food and water ad libitum. They were separated into two groups: N versus HC, each group housed in specific compartments.

In the hypoxic compartment, long-term reduction in the fraction of inspired oxygen (\(F_{iO_2}\)) to 10.5 %, as achieved by mixing air with nitrogen, was used to simulate chronic hypoxia during 6 weeks. Circulating the gas mixture through a water volume into a mixing chamber preserved humidity. \(F_{iO_2}\) was continuously monitored in the hypoxic compartment with an alarm oxygen analyser (GasAlert Extreme; BW Technologies, Calgary, Canada).

For experiments, rats were carefully taken out of their cage and then anaesthetized in a Plexiglass box flushed by air containing 3 % isoflurane. Throughout the experiment, anaesthesia was maintained by continuous inhalation of a gas mixture containing 1.5 % isoflurane delivered in a facemask.

**Experimental set-up**

The experimental set-up was described elsewhere [7]. Briefly, the anaesthetized rat was placed in a supine position into a superconducting magnet (4.7 Tesla; Bruker, Ettlingen, Germany) so that the right segment of the lower limb was centred inside a 30 mm diameter \(^1\)H Helmotz imaging coil tuned to the proton frequency (200 MHz). A circular homebuilt transmit–receive \(^{31}\)P probe (18 mm) was placed horizontally under the gastrocnemius and tuned to the phosphorus frequency (81.1 MHz). Proton shimming was achieved locally in a 20 \(\times\) 20 \(\times\) 20 mm\(^3\) voxel by using the water proton resonance, with typical widths at half-heights of 30–35 Hz.

Before measurements in contracting muscles, a proton MR image was acquired axially by using the \(^1\)H coil to verify muscle group position and to improve field homogeneity.

The plantar flexor muscles group was stimulated electrically (Compex 2 stimulator; Ecublens) directly with transcutaneous electrodes located at the knee and heel levels. For the study of moderate intensity of exercise, the current intensity was set at 6 mA in N rats as well as in HC rats.

A total of 30 \(^{31}\)P spectra (100 \(\mu\)s rectangular pulse, 60° flip angle in the middle of the coil; 64 accumulations; 2.8 s recovery time; 3.3 kHz bandwidth; 1024 data points) were acquired in 180 s blocks throughout the experimental protocol.

Two \(^{31}\)P-MR spectroscopy spectra were acquired to assess the level of muscle PCR at rest. Then, during a warm-up period, muscle stimulation was increased progressively and hypoxia was gradually initiated by \(F_{iO_2}\) reduction of inhaled gas (21–9 % \(O_2\)). No measurements were taken into account for the subsequent analysis until contraction and the spectra reached a steady state. Typically this transitory period lasted approx. 40 min (13 spectra).

Once the hypoxic (9 % \(O_2\)) steady state was reached and maintained for 15 min, a first switch to air for additional 15 min (five spectra) followed by a second switch to a lower electrical stimulation (five additional spectra) provided modulations around the studied steady state of PCR and muscle work rate. The elasticity coefficient of the producer and consumer blocks, flux-control coefficients and the direct effect of \(O_2\) on producer (see below) were determined around this steady state.

**Figure 1** The defined modular system and elasticities quantified by control and regulation analyses

The energetic system of contraction in skeletal muscle in vivo was defined as two modules: the ATP/PCr-producer and the ATP/PCr-consumer. The consumer module embodies all the cellular ATPases that hydrolyse ATP. The producer module embodies all systemic and cellular steps that allow ATP production in active myocytes.

**System variables**

The conceptual system of muscle energetics used here is composed of two modules: the ATP/PCr-producer and the ATP/PCr-consumer (Figure 1). The analysis of the system requires the measurements of two variables: the intermediate and the flux through each module; the latter is equal to the overall flux through the system under steady-state conditions. The representative ‘intermediate’ in the system was PCR concentration, as assessed by \(^{31}\)P-MR-spectroscopy; muscle work rate represented the ‘flux’ as assessed by plantar flexion force exerted on a pedal [7].

Concentrations of metabolic intermediates were assessed from MR-spectroscopy spectra. MR-spectroscopy spectra were routinely deconvolved into Lorentzian lines (Igor Pro Wave metrics, Lake Oswego, OR, U.S.A.). The phosphocreatine, \(P_i\), and \(\beta\)-ATP peak areas were calculated and were converted into concentrations assuming [ATP] = 8.2 mM.

Contraction resulting from muscle electrical stimulation was measured with a homebuilt ergometer consisting of a foot pedal connected to a hydraulic piston. A hydraulic circuit filled with water connected the piston to a force transducer (ref. no. MLT0699; AD Instruments PowerLab Systems) that was placed outside the magnet. Changes in pressure induced by pedal stroke were recorded at 200 Hz. The magnitude of strokes was computed as a function of time (Igor Pro; Wavemetrics) to provide the work rate (contraction) signal.

**Modular control analysis**

The experimental set-up made it possible to quantify concomitant changes in steady levels of PCR and contraction during modulations around the studied steady state called ‘reference’. Following the principles of Metabolic Control Analysis, the modulation by acute hypoxia of steady levels of PCR and contraction made it possible to determine two parameters critical for our understanding of integrative regulation of the modular system by acute hypoxia: (i) the consumer elasticity (\(\varepsilon_c\)) and (ii) the role of oxygen as an external effector of the producer module. The consumer elasticity was determined by relative change in contraction divided by relative change in PCR, induced by acute hypoxia. The direct effect of acute hypoxia on the producer was determined by the relative change in contraction divided by the flux-control coefficient of the producer (see below). The modulation by electrical stimulation allowed the quantification of the producer elasticity (\(\varepsilon_p\)). The producer elasticity was determined by the relative change in contraction divided by the relative change in PCR, induced by decrease in electrical stimulation. Summation and connectivity theorems allowed the
Hypoxic regulation of muscle ATP turnover: top-down analysis

Figure 2 Typical recordings in an HC rat

Recordings were obtained after the transitory period (results not shown). Lowest peaks of PCr (front) indicate the period during which the HC rat inhaled 9 % O2; correspondingly contraction was low as indicated by pedal strokes due to plantar flexion. Highest peaks of PCr (back) were obtained during the low-electrical stimulation period; correspondingly contraction was low. The ‘reference’ steady state is characterized by PCr peaks of intermediate height and intermediate level of contraction. Elasticities were calculated from averaged pedal strokes and spectra.

calculation of flux-control coefficients from the experimental elasticities. The flux-control coefficient of the producer \( C_p \) was calculated as \( \frac{\varepsilon_c}{\varepsilon_c - \varepsilon_p} \); the flux-control coefficient of the consumer \( C_c \) equals \( \frac{\varepsilon_p}{\varepsilon_p - \varepsilon_c} \).

Enzymatic analysis

Once all the non-invasive measurements were performed, the animal was killed by the injection of a lethal dose of pentobarbital. A portion of the rat soleus muscle (∼80 mg) was rapidly excised with scissors and immediately cooled in an ice-cold buffer consisting of 25 mM sucrose, 5 mM Mops and 0.2 mM EDTA. Whole tissue homogenates were prepared in the same buffer by hand homogenization using a Polytron (PT 1200E; Kinematica) at 4 °C. An aliquot of the homogenate (150 μl) was used for measuring Cox (cytochrome c oxidase) activity, by following the decrease in attenuance at 550 nm in a spectrometer (Cary 50 UV–Vis spectrophotometer; Varian) with chemically reduced horse heart cytochrome c as an electron donor. The reaction mixture contained (in mM): 100 KCl, 40 saccharose, 10 KH₂PO₄, 5 MgCl₂·H₂O, 1 EGTA and 0.1 % (w/v) BSA (pH 7.2). The reaction was started by addition of reduced cytochrome c (50 μM). Cox activity was expressed in μmol/min per g of protein.

Blood analysis

The Hct (haematocrit) was determined using blood collected from rat heart and centrifuged in a micro-capillary tube.

RESULTS

HC rats exposed to 10.5 % O₂ during 6 weeks had higher Hct than control N rats (52 ± 3 % versus 42 ± 3 %, \( P < 0.05 \)), thus indicating improved oxygen transport capacity. HC rats had also lower Cox activity (250 ± 12 versus 343 ± 27 μmol/min per g, \( P < 0.05 \)) possibly indicating a lower maximal oxidative capacity in the soleus muscle. Taken together, these results indicate that our rats exposed for 6 weeks to 10.5 % O₂ were effectively conditioned.

The present elasticity analysis was based on simultaneous recordings of steady levels of contraction and PCr during a steady-state exercise modulated by acute hypoxia (9 % O₂) and slight decrease in electrical stimulation as illustrated in Figure 2. Figure 3 provides a comparative overview of averaged changes in PCr and contraction due to modulations by 9 % O₂ and decrease in electrical stimulation in N and HC rats. Solid lines represent the effect of each modulation around the studied steady state called ‘reference’. As quantified below by the elasticity analysis, Figure 3 shows similar slopes (solid lines) in HC and N rats in response to each modulation. It is also worth noting that acute hypoxia (9 % O₂) induced a lower decrease in both PCr and contraction in HC rats when compared with N rats.

Averaged elasticities of the ATP/PCr-producer and ATP/PCr-consumer module in each population (HC versus N) are shown in Table 1. One main finding of the present study was the absence of difference between elasticities in HC rats and N rats.

<table>
<thead>
<tr>
<th></th>
<th>N rats</th>
<th>HC rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elasticity coefficients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP/PCr-producer</td>
<td>(-1.6 ± 1.4(20))</td>
<td>(-1.7 ± 0.8(17))</td>
</tr>
<tr>
<td>ATP/PCr-consumer</td>
<td>(0.8 ± 0.6(28))</td>
<td>(1.1 ± 0.7(23))</td>
</tr>
<tr>
<td>Flux-control coefficients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP/PCr-producer</td>
<td>(34 ± 23 % (20))</td>
<td>(35 ± 17 % (17))</td>
</tr>
<tr>
<td>ATP/PCr-consumer</td>
<td>(66 ± 23 % (20))</td>
<td>(65 ± 17 % (17))</td>
</tr>
<tr>
<td>Direct effect of acute hypoxia on the ATP/PCr-producer</td>
<td>(-44 ± 23 % (20))</td>
<td>(-31 ± 18 %* (17))</td>
</tr>
</tbody>
</table>

Table 1 Coefficients determined by the control and regulation analyses

Values are means ± S.D. The number of experiments is indicated in parentheses. See text for the definition of the calculated coefficients. *\( P < 0.05 \).
Figure 3  Effects of modulations on PCr and contraction

Lines indicate effects of acute hypoxia and lowering electrical stimulation on steady values of PCr and contraction in N rats (squares) and HC rats (circles). Therefore slopes provide a rough illustration of elasticities. Slopes look similar in N and HC rats, as confirmed by the actual calculation of elasticity coefficients (Table 1) thanks to control analysis. Arrows show the more marked effect of acute hypoxia in N rats, studied here by tools of regulation analysis.

Flux-control coefficients calculated from these experimental elasticities are reported in Table 1. A very similar control distribution was quantified in N and HC rats. In the present study where moderate work intensity was stimulated, the control over ATP turnover and contraction was dominantly on the consumer (\(C_c = 65\%\)). Yet, since the producer had substantial control over contraction (\(C_p = 35\%\)), any external effector that can affect directly the producer activity is expected to alter contraction to some extent. Acute hypoxia (9\% \(O_2\)) was just the effector whose direct effect on the producer was quantified here. Since contraction was less inhibited by acute hypoxia in HC rats, while the producer, which is the target of acute hypoxia, had similar control over contraction, a lower direct effect of acute hypoxia on the producer must be concluded. Accordingly, the quantified direct effect of acute hypoxia on the ATP/PCr-producer module in HC rats was only 70\% of the direct effect in N rats (Table 1).

DISCUSSION

The present study demonstrates that, in moderately contracting skeletal muscle, internal regulatory features of ATP turnover as determined here by elasticity coefficients in vivo are likely not affected by long-term exposure to 10.5\% \(O_2\). In agreement with previous results, e.g. [6], we additionally observed a lower impact of acute hypoxia (9\% \(O_2\)) on contraction in HC animals, which can be viewed as a positive adaptation to chronic hypoxia. The application of regulation analysis indicates that in the absence of change in internal regulation of ATP turnover, this adaptation in HC rats is fully explained by a lower direct effect of ambient hypoxia on the producer.

There is growing appreciation of the concept that an improved understanding of complex regulatory processes could be gained from integrative determination of modular elasticity coefficients in intact organs. By conceptually defining heart energetics as a two-module system, the ATP/PCr-producer and the ATP/PCr-consumer, Diolez et al. [9] highlighted the routes taken by regulatory effects of calcium in the beating heart. Korzeniewski et al. [10] incorporated elasticity coefficients into the Proportional Activation Approach and demonstrated that both ATP/PCr-producer and ATP/PCr-consumer modules are directly activated to a similar extent during physiological activation of the heart by adrenaline. As regards skeletal muscle, Jeneson et al. [17] and recently Arsac et al. [7] proposed theoretical and experimental approaches respectively for the assessment in vivo of elasticity coefficients of ATP/PCr-producer and ATP/PCr-consumer modules. Although such methods are usually crude, they have been demonstrated to significantly strengthen the field of physiological regulations in skeletal muscle [16]. Since in the present study, the producer elasticity reached similar values in N and HC rats, we conclude that hypoxic conditioning has no effect on the reactivity of oxidative phosphorylation to energetic intermediates (ADP, ATP, \(P_i\), PCr, Cr and the phosphorylation potential) during moderate exercise. In other words, the substrate feedback control of tissue respiration is likely unaltered by chronic hypoxia. Although our approach actually did not evaluate variations in all these key regulators but focused on PCr as representative, the degree to which PCr is broken down in our conditions fits well with changes of the energy charge of the cell [2,18,21,22]. Force output, as a surrogate of ATP turnover, was the other critical variable measured in the present approach. For reliable MCA (Metabolic Control Analysis) one should assume that contractile efficiency is not affected by hypoxia, incidental fatigue due to prolonged work or stimulation intensity. In humans cycling at submaximal intensities, no change in efficiency was observed at sea level, acute and chronic altitude of 4300 m [23]. In addition, in electrically stimulated canine gastrocnemius under control, ischaemic and hypoxaemic conditions, there was clearly no effect of a lower \(O_2\) delivery on efficiency, as indicated by proportional decrease in muscle force output and \(O_2\) uptake [24]. In steady states prolonged for hours (>2 h; results not shown), we noted no decline in force...
output or in PCr concentration, even at the highest (submaximal) intensities used in these experiments. We concluded that electrical stimulation intensities generate non-fatiguing conditions and that efficiency therefore is unlikely to change with respect to time under our conditions. At last, an obvious candidate for a change in efficiency due to different stimulation intensities is a shift in the recruitment of oxidative and glycolytic fibres. Our previous $T_2^*$ (transverse relaxation time constants)-weighted images (based on $^1$H-MR imaging) indicated that fibres of the mixed gastrocnemius are recruited at various electrical intensities [7]. Furthermore, muscle pH during the periods of interest reached $6.97 \pm 0.04$ in N rats and $6.94 \pm 0.07$ in HC rats with no value below 6.87, thus indicating that ATP synthesis is fully, or at least to a large extent, oxidative in every experiment.

Although the producer elasticity is a critical parameter that likely reveals kinetic or thermodynamic ‘driving functions’ for mitochondrial metabolism, the comprehensive understanding of ATP turnover regulation requires paying attention to the reactivity of ATP/PCr consumption as well. In the present study, we obtained similar consumer elasticities in N and HC rats. Therefore similar $\varepsilon_p$, as well as similar $\varepsilon_c$ in both populations suggest that internal kinetic functions involved in the regulation of muscle ATP turnover in vivo are essentially not altered in HC rats, as compared with N rats.

The absence of changes in elasticity to the intermediates of ATP production and ATP consumption in hypoxic animals is not documented elsewhere in the literature. Our results in normoxic animals can yet be compared with experiments based on electrically stimulated skeletal muscle of animals in situ. Hogan et al. [2] obtained concomitant recordings of contraction and PCr levels in electrically stimulated canine gastrocnemius in situ at two levels of stimulation and under normal and low O$_2$ conditions. So, the elasticity of conceptual producer and consumer modules in their normoxic animals can be calculated from concomitant changes in oxygen uptake and [PCr] in response to hypoxaemia and electrical stimulation. The calculated values of $\varepsilon_p = 1.1$ for the producer elasticity and $\varepsilon_c = 1.2$ for the consumer elasticity are consistent with the present quantification in N rats (Table 1). These similarities in elasticity coefficients obtained in intact muscle in previous studies [2,7] as well as in the present study indicate that, although the proposed experimental methods might be crude, elasticity coefficients can be determined in intact muscle with fine consistency. Therefore we exclude that HC rats might have altered elasticities undetected by our present approach and point definitively to the absence of altered in internal regulatory features of ATP turnover and contraction in our HC rats.

The theory of metabolic control analysis has shown that control over the flux through a given pathway can be distributed over many steps in the pathway and that the degree of control of any given step can be quantified by its flux-control coefficient [15]. In the present study, the control over ATP turnover and contraction derived from elasticity values was effectively distributed between the two modules in each population (Table 1). The quantification of flux-control coefficients highlights similar $C_p,a$ amounting to 35% in N and HC rats. The critical consequence for our understanding of regulation by O$_2$ of muscle energetics is that any direct effect of O$_2$ on the producer activity will be transmitted to contraction with similar ‘strength’ (35%) in N and HC rats. Therefore similar control strength over the flux by the producer module in N and HC rats has to be analysed in concert with the overall effect of 9% O$_2$ on contraction (the so-called global effect in the framework of regulation analysis). This global effect was only $-9 \pm 6$% in HC rats but amounted to $-17 \pm 14$% in N rats (Figure 3). Since this difference in global effect cannot be explained by different control by the producer over contraction, it is fully explained by a lower direct effect of 9% O$_2$ on the producer module, amounting to $-31$% in HC rats but $-44$% in N rats ($P < 0.05$). It is therefore concluded that the target of hypoxic conditioning in the integrated energetic system of contraction in vivo is not the sensitivity of ATP/PCr-producing processes to energetic intermediates — as reflected by elasticity coefficients — but the sensitivity of these processes to ambient O$_2$. This is a critical advantage of regulation analysis to quantify direct effects of external effectors on a metabolic pathway. The 30% lower direct effect of O$_2$ on mitochondrial ATP/PCr production quantified here in HC rats was obtained after 6 weeks at 10.5% O$_2$ in initially healthy rats. Different conditioning patterns may generate different strength of adaptation. Similarly, any initial pathology in the cardiorespiratory system of the animal may result in more or less severe change in the direct effect of acute hypoxia. Modular regulation analysis likely has the potential to quantify these particular integrated responses.

Our study was not designed to specifically identify the very mechanisms responsible for the lower sensitivity to ambient O$_2$ of ATP/PCr production in hypoxic rats. In our conceptual definition of the two-module system, the producer module includes all the steps from O$_2$ and substrates uptake to phosphorylation. So, the concept of direct effect of O$_2$ quantified here embodies integrative properties of cellular steps as well as steps involved in the transfer of O$_2$ from lung to mitochondria. The increase in Hct in HC rats (52 versus 42%) indicates improved O$_2$ transport capacity; the lower Cox activity might indicate down-regulations in cell metabolism. Yet, these responses are not sufficient to assess to what extent the lower sensitivity of ATP production to O$_2$ is due to adaptations in systemic O$_2$ delivery [23] or due to cellular adaptations to chronic hypoxia [25]. A better understanding might come from in vivo assessments of muscle $pO_2$, as achieved for instance by proton MR spectroscopy of deoxymyoglobin [26]. Unfortunately, due to the low sensitivity of magnetic resonance, we are not aware of deoxymyoglobin assessments in small muscles of animals.

An interesting relationship to other $^3$P-MR approaches could be mentioned. As force is supposed to reflect the oxidative ATP rate (called $J$) in our conditions and assuming (reasonably, in this largely aerobic exercise) usual linearity of $J$ to PCr, the location of the hypoxia point in N and HC rats in Figure 3 corresponds to a decreased $\Delta J/A$Pcr, which would correspond to a reduced rate constant (increased time constant) of PCr kinetics during onset or offset of exercise (recovery from stimulation). Such a decrease in PCr recovery rate constant has been noted in a variety of experimental and pathological muscle-hypoxic states, and amounts to approx. $-50$% in healthy humans inhaling 10% O$_2$ [27]. This value could be compared with $-44$%, the direct effect of hypoxia on the producer assessed here by regulation analysis in N rats. We are not aware of assessment of PCr recovery rate constants in HC animals inhaling hypoxic mixtures. Finally, it is worth noting that PCr recovery rate (or time) constants say nothing about the elasticity of ATP/PCr production and ATP/PCr consumption. Elasticities determine the regulation of ATP turnover in contracting muscle, are uniquely determined by MCA and may thus be used to uncover the origin of the observed dysfunctions under hypoxia conditions [8].

In conclusion, the present study provides a quantitative approach to the effects of acute and long-term hypoxia on muscle energetics. In vivo assessments of elasticity coefficients and direct effect of O$_2$ on ATP production illustrate how internal and external regulation of ATP turnover and contraction can be uncovered. We anticipate that the approach is useful to detect dysfunctions or improved functions at system levels [28] and brings new tools for integrative physiology [29].
FUNDING

This work was supported by Centre National de la Recherche Scientifique (CNRS), Université Victor Segalen (Bordeaux 2), Conseil Régional de la Région Aquitaine and Institut Fédéral de Recherche (IFR)4, Bordeaux, France.

REFERENCES


Received 16 December 2008/13 February 2009; accepted 19 February 2009
Published as BJ Immediate Publication 19 February 2009, doi:10.1042/BJ20082385

© The Authors Journal compilation © 2009 Biochemical Society