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Metabolic underpinnings of the paradoxical net phosphocreatine resynthesis in contracting rat gastrocnemius muscle

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Abstract

Net phosphocreatine (PCr) resynthesis during muscle contraction is a paradoxical phenomenon because it occurs under conditions of high energy demand. The metabolic underpinnings of this phenomenon were analyzed non-invasively using ^{31}P-magnetic resonance spectroscopy in rat gastrocnemius muscle ($n = 11$) electrically stimulated (7.6 Hz, 6 min duration) in situ under ischemic and normoxic conditions. During ischemic stimulation, [PCr] initially fell to a steady state ($9 \pm 5\%$ of resting concentration) which was maintained for the last 5 min of stimulation, whereas isometric force production decreased to a non-measurable level beyond 3 min. Throughout normoxic stimulation, [PCr] and force production declined to a steady state after respectively 1 min ($5 \pm 3\%$ of resting concentration) and 3.25 min ($21 \pm 8\%$ of initial value) of stimulation. Contrary to the observations under ischemia, a paradoxical net PCr resynthesis was recorded during the last 2 min of normoxic stimulation and was not accompanied by any improvement in force production. These results demonstrate that the paradoxical net PCr resynthesis recorded in contracting muscle relies exclusively on oxidative energy production and could occur in inactivated fibers, similarly to PCr resynthesis during post-exercise recovery.

Keywords: Phosphorus-31 magnetic resonance spectroscopy; Rat skeletal muscle; Bioenergetics; Metabolism; Fatigue; Oxygen

1. Introduction

Intramuscular concentration of phosphocreatine (PCr) is under the control of creatine kinase (CK), which reversibly transfers high energy phosphate from PCr to ADP to form ATP via the reaction: $PCr + ADP + H^+ \leftrightarrow ATP + creatine$.

In the early stage of muscle contraction, the PCr-CK system is commonly considered as a temporal energy buffer, which maintains the ATP pool highly charged [1,2]. At the transition from rest to exercise, [ATP] remains unchanged [3] although ATP demand may increase more than 100-fold and exceed ATP production [4,5]. This [ATP] homeostasis is allowed by a rapid net breakdown in PCr, which is the only source of energy contributing to ATP regeneration [1,2]. With continuation of muscle activity, glycolysis and oxidative phosphorylation progressively carry out ATP regeneration and PCr concentration stops to decline to reach a steady state. At this stage, the pool of PCr behaves as a shuttle for the transport of high-energy phosphates between the sites of production and utilization of ATP [6], and

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the PCr-CK system functions as a spatial energy buffer [1,2].

Net PCr resynthesis is usually observed during post-exercise recovery, i.e. as soon as the muscle ceases to produce any mechanical work [1,7,8]. Surprisingly, some studies have previously described a net PCr resynthesis in rat gastrocnemius muscle during exhaustive muscle contraction [9,10]. This phenomenon is paradoxical because it occurs under a situation of high ATP demand, hence suggesting that muscular energy would promote PCr resynthesis instead of being used to produce force. This phenomenon remains poorly investigated, with the exception of a study showing that the net PCr resynthesis during muscle contraction might occur in inactivated fibers as a result of muscle fatigue [10]. In particular, the metabolic events accounting for PCr resynthesis during muscle contraction have never been documented.

The purpose of the present study was to determine the metabolic underpinnings of PCr resynthesis in rat gastrocnemius muscle. In theory, anaerobic glycolysis and oxidative phosphorylation are the two major sources of ATP for PCr resynthesis during contraction. In order to discriminate which contribution (anaerobic vs. oxidative) was predominant during this paradoxical process, rat gastrocnemius muscles were stimulated in situ under conditions leading to PCr resynthesis during contraction and metabolic changes under normoxic and ischemic conditions were analyzed using ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS).

2. Materials and methods

2.1. Animal care and feeding

Eleven male Wistar rats (CERJ, Le Genest St Isle, France) weighing 350–375 g were used for these experiments, following the guidelines of the National Research Council Guide for the care and use of laboratory animals, and the French Law on Animal Handling and Protection. Rats were housed in an environment-controlled facility (12/12 h light–dark cycle; 22°C) and received water and standard rat food ad libitum until the time of experiment. At the end of the experiments, animals were immediately euthanasied by an intracardiac injection of pentobarbital sodium.

2.2. Hind limb surgical preparation

General anesthesia was induced with an intraperitoneal injection of pentobarbital sodium (50 mg kg^{-1} body weight), and maintained throughout the experiment by repeated administrations of anesthetics (10 mg kg^{-1} body weight, every 30 min) through an intraperitoneal catheter. The left hind limb was surgically prepared for in situ sciatic nerve stimulation of the gastrocnemius muscle. During the experiment, the gastrocnemius muscle was not exposed in order to secure physiological conditions. The Achilles tendon was exposed and removed from the foot at the site of the calcaneus bone attachment, leaving intact the neurovascular supply of the muscle. The distal part of the gastrocnemius tendon was attached to a home-built force transducer via an inextensible silk thread. Through a small incision made at the hip level, the left sciatic nerve was exposed in its gluteal course between the quadratus femoris and the caudofemoralis muscles, and carefully cleared of connective tissue. A home-built bipolar electrode connected to an electrical stimulator (SI-10, NARCO, USA) was placed around the sciatic nerve, which was cut proximally to the electrode attachment. Sciatic nerve connected to the bipolar electrode was put back in place and the incision was closed.

In order to ensure ischemic conditions, the femoral artery and vein were isolated at the groin level, between pectineus and iliacus muscles. A rigid plastic cylinder (8 mm length, 5 mm inner diameter) with a longitudinal slit was placed around the femoral artery and vein. A water-inflatable rubber balloon was inserted into the cylinder and positioned beside the femoral artery and vein. Reversible leg ischemia was accomplished by inflating the rubber balloon to a predefined volume.

The rat was placed backwards in a custom-made Perspex cradle integrating a warm water heating pad. Body temperature (typically 35–36°C) was monitored throughout the experiment using a rectal probe. The left leg was firmly immobilized by securing the foot with straps and by inserting a non-magnetic brass pin into the tibia head. In that position, the belly

of the gastrocnemius muscle was located above the ^{31}P-MRS surface coil. At rest, muscle was passively loaded (typically 1.3–1.5 N) by adjusting the position of the force transducer in order to give maximum isometric twitch tension in response to supramaximal square wave pulses (1–10 V; 1 ms duration) delivered to the sciatic nerve. At this stage, the cradle was inserted into the magnet.

2.3. Experimental protocol

The experimental protocol consisted of 6 min of rest, 6 min of isometric contractions, and 30 min of recovery. Isometric contractions were electrically induced at 7.6 Hz with supramaximal square-wave pulses (1–10 V, 0.2 ms duration), under normoxic ($n=6$) or ischemic ($n=5$) conditions. In the ischemic group, blood occlusion (23.5 min duration) was induced at rest and into the magnet, 15 min before the beginning of stimulation. It has been shown previously that this duration of ischemia was long enough to deplete muscle oxygen stores [11]. Blood occlusion was released 2.5 min after the end of stimulation (Fig. 1).

2.4. Force measurements

Force measurements were conducted with a home-built force transducer, which was linear from 0 to 10 N. During muscle stimulation, the electrical signal coming out from the force transducer was amplified (reference: 13-4515-50, Gould, USA), then converted to a digital signal and processed on a personal computer using ATS software (SYSMA, France). Isometric force production (in Ns) was calculated every 15 s of stimulation by integrating the isometric tension (in N) relative to time (in s).

2.5. MR spectroscopy and data processing

^{31}P-MRS investigations were performed in a horizontal superconducting magnet (Brüker 47/30 Biospec system, Karlsruhe, Germany) operating at 4.7 Tesla. MR data were collected with a home-built ^{31}P-MRS surface coil (10×14 mm). Magnetic field homogeneity was optimized by monitoring the water signal until the width of the water peak at half height was below 0.25 ppm. ^{31}P-MR signal was acquired at 81 MHz following a 20 μs radiofrequency pulse applied with a repetition time (TR) of 2.4 s. MR data acquisition was gated to the stimulation in order to record signal between consecutive contractions and then reduce motion artifacts due to contraction. Free induction decays (FIDs; 12 scans, 4000 Hz sweep width, 512 data points collected) were continuously recorded in 30 s blocks throughout the experimental protocol, i.e. during 6 min before stimulation (rest), during stimulation (6 min) and during 30 min after stimulation (recovery). FIDs were transferred to an IBM RISC 6000 workstation and processed using the NMR1 spectroscopy processing software (New Methods Research, USA). After deconvolution to a line broadening of 15 Hz and application of zero filling (2 K), FIDs were Fourier transformed into spectra and baseline correction was performed as previously described [12]. Signal areas corresponding to phosphorylated metabolites were measured after curve fitting to a Lorentzian shape function [12], and corrected for magnetic saturation effects using fully relaxed spectra collected at rest with a TR of 20 s duration. Intracellular pH (pH_i) was calculated from the chemical shift of P_i relative to PCr (−2.45 ppm) as previously described [7]. Absolute concentrations of phosphorylated metabolites were expressed relative to a resting β-ATP concentration of 5.8 mM reported from fluorimetrical measurements in the same muscle [13]. Time points for the time course of [PCr] were assigned to the midpoint of the acquisition interval.

2.6. Statistics

All results are presented as mean ± S.E.M. Statistical differences were tested within the stimulation protocol using two-tailed Student's *t*-test for paired observations, and between stimulation protocols using one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls post hoc test for multiple comparisons. The level of significance was set at $P<0.05$.

3. Results

3.1. Time-dependent changes in phosphorylated metabolites and intracellular pH

Typical ^{31}P-MRS spectra recorded on the rat gastrocnemius muscle are presented in Fig. 2. No changes in [PCr], [ATP] and pH_i were measured throughout the resting period either under normoxic or under ischemic conditions. At the end of the rest period, [PCr], [ATP] and pH_i did not differ significantly between normoxic and ischemic conditions (Figs. 3–5).

3.1.1. PCr

PCr was rapidly hydrolyzed at the onset of stimulation in both protocols (Fig. 3). The initial rates of hydrolysis (VPCr$_{start}$) were not significantly different between normoxic (77.4 ± 8.3 mM min^{-1}) and ischemic (63.3 ± 7.6 mM min^{-1}) stimulations (Table 1). During normoxic stimulation, [PCr] fell to reach a minimal value at 0.9 ± 0.5 mM ($5 \pm 3\%$ of resting value) after 1 min of stimulation (Fig. 3). This level remained fairly constant until the 4th min of stimulation. Surprisingly, [PCr] increased significantly at this time at a rate of 1.5 ± 0.2 mM min^{-1} (Table 1), to reach 2.9 ± 0.6 mM ($17 \pm 1\%$ of rest) at the end of the stimulation period (Fig. 3). At the start of post-stimulation recovery, the resynthesis of PCr occurred at a rate of 2.9 ± 0.3 mM min^{-1} (Table 1). During ischemic stimulation, time-dependent changes in [PCr] were different from those recorded during normoxic stimulation (Fig. 3): [PCr] fell to reach a minimal concentration at 1.9 ± 0.2 mM ($9 \pm 3\%$ of resting concentration) after 1 min of stimulation but, in contrast to normoxic conditions, this concentration remained fairly constant until the end of the stimulation period, with no PCr resynthesis during stimulation (Fig. 3). With the cessation of stimulation, no significant PCr resynthesis was measured throughout post-stimulation recovery under ischemic conditions (2.5 min duration). PCr resynthesis began during normoxic recovery, 30 s after ischemia was released (Fig. 3), at the initial rate of 2.2 ± 0.8 mM min^{-1}.

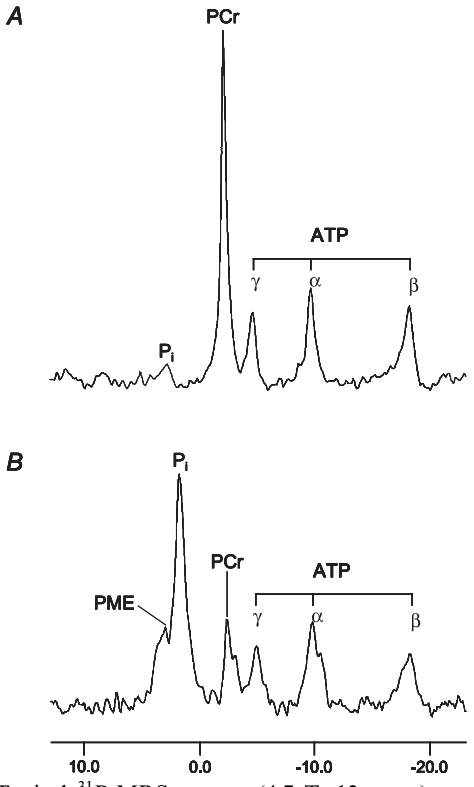

Fig. 2. Typical ^{31}P-MRS spectra (4.7 T, 12 scans) recorded on the gastrocnemius muscle from a single rat during the normoxic protocol, at rest (A) and at the end of 6 min stimulation at 7.6 Hz (B). Abbreviations for signal assignment (in ppm, part per million) are PME (phosphomonoester), P_i (inorganic phosphate), PCr (phosphocreatine) and γ-, α- and β-resonances of ATP.

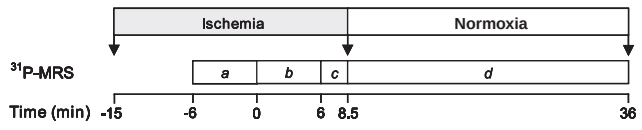

Fig. 1. Experimental design for the ischemic protocol. Ischemia is induced 15 min before muscle stimulation and released 2.5 min after muscle stimulation. ^{31}P-MRS measurements are performed during rest (a), muscle stimulation (b), ischemic recovery (c), and normoxic recovery (d).

3.1.2. ATP

In the early stage of stimulation, [ATP] fell during both protocols to reach a steady state after 1.25 min of stimulation (Fig. 4). This steady state was not significantly different between normoxic (3.9 ± 1.2 mM) and ischemic (3.8 ± 0.6 mM) stimulations. Under normoxic conditions, the steady state was maintained until 4.25 min of stimulation, but decreased slightly afterwards to reach 2.5 ± 0.6 mM ($41 \pm 11\%$ of resting value) at the end of the stimu-

lation period. Under ischemic conditions, the steady state was maintained until the end of stimulation when [ATP] reached 3.9 ± 0.6 mM (64 ± 9% of resting value). [ATP] measured at the end of the ischemic stimulation period was significantly larger (more than 1.5-fold) than under normoxic stimulation.

3.1.3. Intracellular pH

In both normoxic and ischemic protocols, pH_i decreased from the beginning of stimulation, and reached a steady state after 1.25 min of stimulation (Fig. 5). This steady state was maintained until the end of stimulation, where pH_i was not significantly different between normoxic (6.22 ± 0.06) and ischemic (6.17 ± 0.03) protocols.

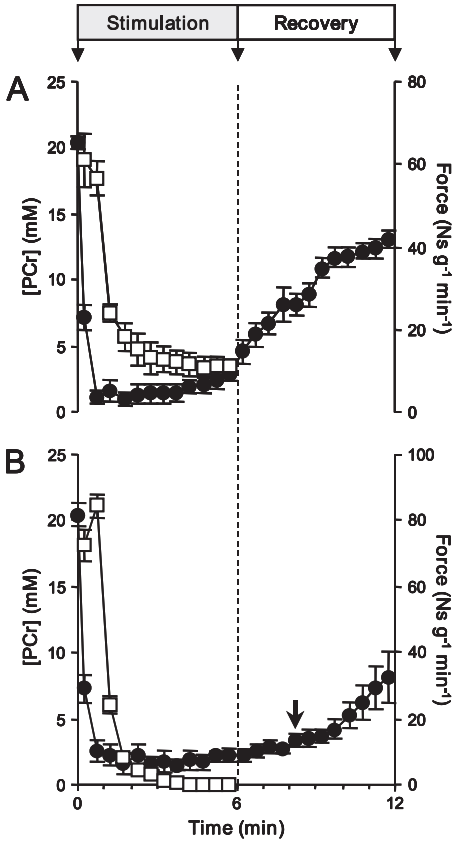

Fig. 3. Time-dependent changes in PCr concentration (●) and in isometric force production per twitch (□) during and after stimulation of rat gastrocnemius at 7.6 Hz under normoxic (A) and ischemic (B) conditions. For the time-dependent changes in [PCr], the first point indicates the resting value. For the ischemic protocol (B), the black arrow indicates ischemia release. Values are means ± S.E.M.

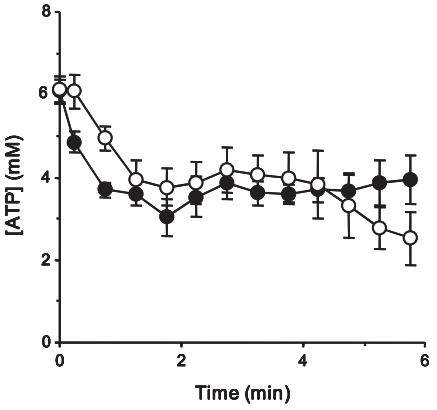

Fig. 4. Time-dependent changes in ATP concentration during stimulation of rat gastrocnemius performed at 7.6 Hz under normoxic (○) and ischemic (●) conditions. Values are means ± S.E.M.

3.2. Time-dependent changes in isometric force production

At the onset of the stimulation period, isometric force production did not differ significantly between normoxic (61.2 ± 6.0 Ns min^{-1}) and ischemic (72.0 ± 4.8 Ns min^{-1}) protocols (Fig. 3). Throughout stimulation, isometric force production decreased dramatically in both protocols: after 3.25 min of normoxic stimulation, it reached a plateau (11.4 ± 3.0 Ns min^{-1}, i.e. 21 ± 8% of initial value) which was maintained until the end of the stimulation period. On the contrary, no measurable force was recorded beyond 3 min of stimulation under ischemic conditions (Fig. 3).

Table 1
Rates of changes in PCr concentration for normoxic and ischemic protocols during stimulation at 7.6 Hz and recovery

	Protocol	
	Normoxia	Ischemia
n	6	5
$\dot{V}PCr_{start}$	77.4 ± 8.3	63.3 ± 7.6
$\dot{V}PCr_{stim}$	0.8 ± 0.3	–
$\dot{V}PCr_{rec}$	2.9 ± 0.3	2.2 ± 0.8

Values are means ± S.E.M. in mM min^{-1}. *n*, number of rats; $\dot{V}PCr_{start}$, rate of PCr hydrolysis at the start of stimulation; $\dot{V}PCr_{stim}$, rate of net PCr resynthesis during normoxic stimulation; $\dot{V}PCr_{rec}$, rate of PCr resynthesis at the start of recovery; for the ischemic protocol, $\dot{V}PCr_{rec}$ was measured after ischemia was released (2.5 min after stimulation cessation).

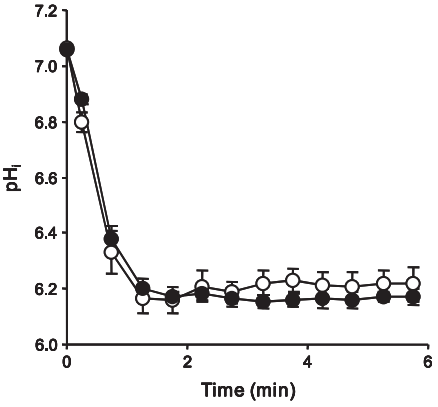

Fig. 5. Time-dependent changes in intracellular pH (pH_i) during stimulation of rat gastrocnemius performed at 7.6 Hz under normoxic (○) and ischemic (●) conditions. Values are means ± S.E.M.

3.3. PCr cost of contraction

PCr cost of contraction, which was calculated at the onset of stimulation as the ratio between the initial rate of PCr breakdown and isometric force production, did not differ significantly between normoxic (1.3 ± 0.1 mM $(Ns)^{-1}$) and ischemic (0.9 ± 0.2 mM $(Ns)^{-1}$) protocols.

4. Discussion

We have investigated for the first time the metabolic underpinnings of the paradoxical net PCr resynthesis during muscle contraction. Two major findings can be emphasized. Firstly, the paradoxical PCr resynthesis that occurred during normoxic contraction was not accompanied by any improvement in force production, hence suggesting that PCr resynthesis occurred in inactivated fibers. Secondly, the paradoxical PCr resynthesis was abolished during ischemic stimulation, hence demonstrating that this phenomenon is a pure oxidative process.

With the start of normoxic stimulation, [PCr] declined rapidly to reach a minimal concentration at 0.9 ± 0.5 mM (i.e. $5 \pm 3\%$ of resting value) after 1 min of stimulation. This minimal concentration remained fairly constant during the following 3 min of stimulation together with [ATP]. These data are in agreement with the concept that the PCr-CK system acts as a spatial energy buffer in the later stage of muscle contraction [1,2]. Indeed, combination of steady states in PCr and ATP concentrations indicates that the pool of PCr behaves as a shuttle for the transport of high-energy phosphates between the sites of production and utilization of ATP [1,2,6].

Surprisingly, this steady state of [PCr] was altered beyond 4 min of stimulation when [PCr] began to increase significantly in a linear manner (1.5 ± 0.2 mM min^{-1}) until the end of the 6 min stimulation period. With regard to the concept of spatial energy buffering, this increase in [PCr] would mean that some ATP is available in excess to promote a net PCr resynthesis during muscle contraction. Such a situation is in opposition with the common view of muscle bioenergetics according to which the rate of ATP production is regulated with a high precision to meet ATP utilization throughout muscle exercise [4,5,14]. The underlying issue is therefore to understand why, under conditions of high energy demand, PCr resynthesis can occur.

The answer could stem from the alteration in the pattern of fiber recruitment throughout fatigue development: rat gastrocnemius muscle is a mixed muscle, which is composed of $7 \pm 1\%$ slow-twitch oxidative fibers (SO), $28 \pm 5\%$ of fast-twitch oxidative glycolytic fibers (FOG), and $65 \pm 5\%$ of fast-twitch glycolytic fibers (FG) [15]. By the end of the exhaustive protocol performed under normoxic conditions, SO fibers (fatigue resistant) could continue to produce force whereas FG and FOG fibers (fast fatigable) could be partially or totally inactivated [10]. In the present study, the paradoxical PCr resynthesis occurred whereas isometric force production was decreased to reach a plateau at $20 \pm 5\%$ of the initial value, i.e. when the muscle was exhausted. The fact that this PCr resynthesis was not accompanied by any changes in isometric force production strongly suggests that PCr resynthesis occurs in inactivated fibers [10]. Our inference is based on the assumption that if a contracting fiber has ATP to spare for PCr resynthesis, it should be using some of that ATP to generate force. This may be so, or it could be that non-metabolic factors prevent this. In any case, further work is needed for a definitive settlement of this issue.

It is of interest to note that during normoxic contraction a slight [ATP] decrease was recorded at the same time when PCr was resynthesized. ATP deple-

tion has been usually reported as a sign of a failure in energy production such as during exhaustive exercise but no PCr resynthesis has ever been reported concomitantly [16,17]. Because the PCr pool is devoted to prevent any dramatic decrease in [ATP] [1,2,6], it is unlikely that ATP was hydrolyzed to promote PCr resynthesis. Instead, the net PCr resynthesis and ATP depletion might occur separately in different types of fibers, PCr resynthesis occurring in inactivated fibers which do not contract anymore and ATP depletion resulting from impaired energy production in fibers still producing force.

The question is now to determine the metabolic origin of PCr resynthesis throughout muscle stimulation. During prolonged exercise under normoxic conditions, glycolysis and oxidative phosphorylation carry out ATP regeneration. It has been demonstrated that glycolysis is closely associated to muscle contraction [18–20], which would explain why glycolysis is immediately stopped at the cessation of contraction [8,17] and therefore does not participate in PCr resynthesis during post-exercise recovery [18]. In addition and in agreement with our results, several lines of evidence including comparative analysis of normoxic and ischemic experiments [7,21] have been reported indicating that resynthesis of PCr during post-exercise recovery is oxygen-dependent. However, the exact nature of this requirement has so far not been elucidated.

In the present study, given that PCr resynthesis during muscle contraction occurred under normoxic conditions, one can assume that anaerobic glycolysis may represent, in addition to oxidative phosphorylation, a potent source of ATP for PCr resynthesis in inactivated fibers.

This hypothesis can, however, be rejected in view of the differences recorded between normoxic and ischemic protocols. Anaerobic glycolysis is the sole metabolic source of ATP during prolonged exercise under ischemic conditions. Our measurements show that no measurable force was recorded beyond 3 min of ischemic stimulation, hence indicating that all muscular fibers were likely inactivated. In contrast to normoxic stimulation, ischemic stimulation was characterized by the absence of any net PCr resynthesis, hence demonstrating that anaerobic glycolysis did not contribute to PCr resynthesis under ischemic stimulation. This result is in agreement with a previous ^{31}P-MRS study in humans showing that post-exercise recovery performed under ischemic conditions was not accompanied by any PCr resynthesis [18]. Consequently, the abolition of the paradoxical PCr resynthesis during ischemic stimulation demonstrates that this phenomenon is a pure oxidative process.

One could argue that the cessation of muscle contraction during ischemic stimulation was caused by energy depletion [5,16] considering that intramuscular glycogen stores, which are the sole source of energy during ischemic contractions, could be fully depleted throughout intensive exercise [22,23]. Our data show that during ischemic stimulation, [ATP] decreased to reach a steady state (at 3.8 ± 0.6 mM) which occurred after 1.25 min of stimulation (i.e. 2 min before muscle contraction was stopped) and which was maintained until the end of the stimulation period. This suggests that [ATP] was not affected by the dramatic decrease in isometric force production. In addition, [ATP] measured at the end of the ischemic stimulation period (3.9 ± 0.6 mM, i.e. $64.1 \pm 8.5\%$ of resting value) was more than 1.5-fold larger than under normoxic stimulation (2.5 ± 0.6 mM, i.e. $41 \pm 11\%$ of resting value), hence indicating that the imbalance between ATP production and ATP utilization was larger during normoxic stimulation than during ischemic stimulation. Nevertheless, such a decrease in [ATP] at the end of normoxic stimulation did not impair isometric force production. Therefore, the cessation of muscle contraction during ischemic stimulation cannot be due to energy depletion, because if such were the case, the reduction in ATP level would have been larger. This impairment of force production could be caused by other factors such as acidosis [24] or impaired excitation–contraction coupling [22].

One key point of the present study was to ensure that the ischemic period before stimulation was long enough (15 min duration) to deplete the oxygen stores. Additional experiments (results not shown) indicate, in agreement with a previous study [11], that an ischemic period at rest longer than 15 min is linked to a net PCr breakdown as a result of complete oxygen depletion. In addition, the absence of PCr resynthesis after stimulation when muscle is kept ischemic (during 2.5 min) further indicates that the ischemic process was efficient. It is noteworthy that

PCr resynthesis did not occur immediately after ischemia was released but after a lag time of 30 s. This lag time has already been reported as a sign of the time necessary for the restoration of blood pressure [11].

During the normoxic protocol, the initial rate of PCr hydrolysis at the onset of stimulation ($V\mathrm{PCr}_{start} = 77.4 \pm 8.3$ mM min^{-1}) was more than 25-fold larger than the initial rate of PCr resynthesis at the onset of post-stimulation recovery ($V\mathrm{PCr}_{rec} = 2.9 \pm 0.3$ mM min^{-1}). This imbalance might appear to be in contradiction with previous studies showing that both initial rates of PCr breakdown and PCr recovery are equal [25,26]. However, it should be kept in mind that this equality is in general observed after moderate exercise during which [PCr] reaches a steady state throughout exercise and the extent of PCr consumption is moderate. Given that these conditions are not fulfilled in the present study, the imbalance between these initial rates could be due to dramatic metabolic changes following high-intensive stimulation. Several factors including an inhibition of CK by hydrogen ions, a significant contribution of glycolysis to ATP production at the end of exercise and a change in contractile efficiency could account for this difference between the initial rate of PCr depletion in exercise and the initial rate of post-exercise PCr resynthesis. It has been shown previously that rabbit muscle CK activity was severely reduced at low pH [24]. Moreover, pH changes measured at the end of the stimulation period indicate a significant contribution of glycolysis to ATP production. On the other hand, changes in contractile efficiency have been reported recently throughout muscle activity [27,28].

Another interesting observation is that the rate of PCr hydrolysis and isometric force production did not differ significantly between both protocols at the onset of stimulation. We found that PCr cost of contraction, which was calculated at this stage of stimulation, did not differ significantly between normoxic (1.3 ± 0.1 mM $(\mathrm{Ns})^{-1}$) and ischemic (0.9 ± 0.2 mM $(\mathrm{Ns})^{-1}$) protocols supporting the hypothesis that oxidative ATP synthesis is negligible in the early stage of normoxic exercise as previously suggested [29]. Indeed, any contribution of oxidative phosphorylation to ATP regeneration during normoxic stimulation would decrease that of PCr hydrolysis. However, one should bear in mind that a significant aerobic contribution to ATP production could have been measured with a higher time resolution and that our inference is based on the assumption that contractile efficiency is not affected by ischemia.

In conclusion, this study provides a novel insight into the bioenergetics of contracting muscle. We have demonstrated that the net PCr resynthesis occurring in contracting muscle under normoxic conditions can be exclusively considered as an oxidative process likely occurring in inactivated fibers, similarly to PCr resynthesis during post-exercise recovery.

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