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Some factors determining the PCr recovery overshoot in skeletal muscle

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Abstract

It has been proposed recently that the phosphocreatine (PCr) overshoot (increase above the resting level) during muscle recovery after exercise is caused by a slow decay during this recovery of the direct activation of oxidative phosphorylation taking place during muscle work. In the present article the factors determining the appearance and size of the PCr overshoot are studied using the computer model of oxidative phosphorylation in intact skeletal muscle developed previously. It is demonstrated that the appearance and duration of this overshoot is positively correlated with the value of the characteristic decay time of the direct activation of oxidative phosphorylation. It is also shown that the size of PCr overshoot is increased by low resting PCr/Cr ratio (what is confirmed by our unpublished experimental data), by high intensity of the direct activation of oxidative phosphorylation, by high muscle work intensity and by low rate of the return of cytosolic pH to the resting value during muscle recovery.

Keywords: Oxidative phosphorylation; Kinetic model; Creatine kinase; Mitochondrial respiration; ADP undershoot; P_i undershoot

1. Introduction

At the onset of exercise in skeletal muscle the PCr concentration decreases from the resting steady-state value to some new value because of a temporary mismatch between the rate of ATP usage and ATP production. After the termination of exercise [PCr] returns to its resting value. During this recovery period [PCr] rises in some cases above its resting value and then slowly returns to the resting level [1–8]. This phenomenon has been named the PCr recovery overshoot. The PCr overshoot is accompanied by P_i undershoot [1], ADP undershoot [3] and, in a consequence, phosphorylation potential overshoot. Sometimes ADP undershoot and P_i undershoot are not accompanied by the PCr overshoot [9–11].

The phosphocreatine overshoot cannot be explained by muscle acidification during work and at the beginning of recovery because low pH shifts the creatine kinase equilibrium toward converting PCr into Cr.

The PCr overshoot has been reported both in glycolytic muscles/muscle fibers after very intensive short-term exercise, where the cytosolic pH decreases significantly during muscle work [5,7], and in oxidative muscles/muscle fibers during intensive exercise, where pH is relatively constant [1,2]. This phenomenon appears several minutes after the termination of exercise [1–3,5] and at least in some cases lasts over 1 h [6].

It is not clear what constitutes the molecular background of the PCr recovery overshoot (as well as P_i undershoot and ADP undershoot). It has been postulated recently [12] that this phenomenon is caused by a slow decay after the termination of exercise of the direct activation of oxidative phosphorylation complexes taking place during skeletal muscle contraction. The parallel direct activation of all oxidative phosphorylation enzymes during muscle work by some factor related to calcium ions, causing e.g. protein phosphorylation, was proposed as the main mechanism of

Abbreviations: Cr, creatine; PCr, phosphocreatine; P_i, inorganic phosphate; On-transient, rest-to-work transition; Off-transient, work-to-rest transition; VO_2, oxygen consumption rate.

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the regulation of oxidative phosphorylation during rest-to-work transition in skeletal muscle [12–14]. This mechanism is able to explain, among others, the steep phenomenological VO_2–[ADP] relationship observed in skeletal muscle during rest-to-work transition [12,13], the much greater maximal oxygen consumption per mitochondria volume in intact skeletal muscle than in isolated muscle mitochondria, skinned fibers and muscle homogenate [14], the asymmetry in the PCr on- and off-transient kinetics [12], the PCr recovery overshoot and P_i recovery undershoot [12], as well as the variability of the kinetic properties of oxidative phosphorylation in different muscles and various experimental conditions [12]. It was also proposed that an increase in the direct activation of oxidative phosphorylation as a result of muscle training is responsible for the greater respiration rate per mg of mitochondrial protein at a given [ADP] in trained muscle than in untrained muscle [12] and for the better ATP/ADP homeostasis (smaller increase in [ADP] at a given increase in VO_2 during rest-to-work transition) in trained muscle than in untrained muscle [15].

In all the cited theoretical studies the dynamic computer model of oxidative phosphorylation developed previously [16,17] and extensively tested by comparison with various experimental data was used. Within this model, the presence of the PCr recovery overshoot (and P_i recovery undershoot) depends on the characteristic decay time τ of the activation of oxidative phosphorylation complexes after the termination of muscle work. The relative activity of oxidative phosphorylation during muscle recovery (scaled to unity in rest) is described by the following equation [12]:

$$m = 1 + (n^p - 1)e^{-t/\tau} \quad (1)$$

where n is the relative activity of ATP usage during work, n^p is the relative activity of oxidative phosphorylation during work, τ is the characteristic decay time of the activation of oxidative phosphorylation and t stands for the time after the termination of exercise. The resting values of the rate constants of particular oxidative phosphorylation steps are multiplied by n^p during exercise and by m during recovery, while the resting value of the rate constant of ATP usage is multiplied by n during work and by 1 during recovery. Thus, it is assumed within the model that the relative activity of oxidative phosphorylation equals 1 at rest, very quickly increases n^p times after the onset of exercise in parallel with an n-fold activation of ATP usage and decays in the exponential way after the termination of exercise, while the activation of ATP usage is switched off instantly at the onset of muscle recovery. The last property is crucial for the appearance of the PCr recovery overshoot (and P_i undershoot)—the overshoot appears because during recovery the ATP-producing block (oxidative phosphorylation) is for some time more active than in rest, while the ATP-consuming block during the entire recovery period is as active as in rest.

In the present study we analyze, using the computer model of oxidative phosphorylation in skeletal muscle [17], some factors/parameters/mechanisms that determine the appearance and the extent of the PCr recovery overshoot. We show that the necessary condition of the appearance of this overshoot is a long enough characteristic time τ of the decay of the direct activation of oxidative phosphorylation during muscle recovery. This time determines also the duration of the PCr overshoot. This overshoot is enhanced by a high intensity of the direct activation of oxidative phosphorylation during muscle exercise, high work intensity and low resting PCr/Cr ratio. On the other hand, the PCr recovery overshoot is delayed, diminished or even masked by a slow return of cytosolic pH during muscle recovery to its resting value. The possible differences in the origin of the PCr overshoot in oxidative muscle and glycolytic muscle as well as the physiological role of this phenomenon are discussed.

2. Theoretical procedures

The previously developed computer model of oxidative phosphorylation in intact skeletal muscle [17] was used in the present theoretical studies. The following enzymes/processes/metabolic blocks are taken into account explicitly within the model: substrate dehydrogenation (hydrogen supply to the respiratory chain including Krebs cycle, glycolysis, glycogenolysis, glucose transport, fatty acid β-oxidation, fatty acid transport and so on), complex I, complex III, complex IV (cytochrome *c* oxidase), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, adenylate kinase, creatine kinase, ATP usage. The time variations of the metabolite concentrations which constitute independent variables (NADH, ubiquinol, reduced form of cytochrome *c*, O_2, internal protons, internal ATP, internal P_i, external ATP, external ADP, external P_i, external protons, PCr) are expressed in the form of a set of ordinary differential equations. The other (dependent) variable values (other metabolite concentrations, thermodynamic forces, etc.) are calculated from the independent variable values. The set of differential equations is integrated numerically. In each iteration step, new values of rates, concentrations and other parameters are calculated on the basis of the corresponding values from the previous step. The Gear procedure was used for numerical integration and the simulation programs were written in the FORTRAN programming language. The complete description of the model is located on the web site http://www.awe.mol.uj.edu.pl/~benio/ (the model of oxidative phosphorylation in intact skeletal muscle).

In the simulations presented in Fig. 6 the version of the model of oxidative phosphorylation containing a simple semi-quantitative kinetic description of anaerobic glycolysis [18] was used in order to study the effect of cytosolic pH on

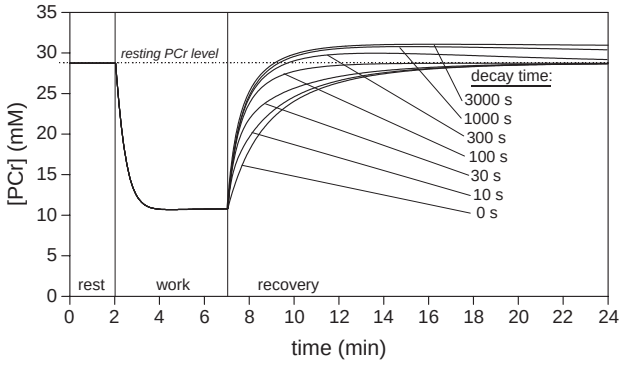

Fig. 1. Simulated effect of the characteristic decay time τ of the activation of oxidative phosphorylation on the PCr kinetics during recovery.

the PCr overshoot. This version is also located on the above web site.

In order to study the effect of particular factors on the PCr recovery overshoot the values of relevant parameters were varied in computer simulations. In the simulations presented in Fig. 1 the value of the characteristic decay time τ of the activation of oxidative phosphorylation (see Eq. (1)) was set to be equal to 0 s, 10 s, 30 s, 100 s, 300 s, 1000 s and 3000 s in subsequent simulations, while n was equal to 50 (intensive muscle work) and p was equal to 0.3 (medium parallel activation of oxidative phosphorylation) in all simulations. In the simulations shown in Fig. 2 the 'standard' rate constant of ATP usage at rest was unchanged (line a), doubled (line b) or increased four times (line c). In order to set the same ATP usage during muscle work in different simulations, the rate constant of ATP usage at the onset of exercise was multiplied by 50 (line a), 25 (line b) or 12.5 (line c). τ was equal to 3000 s (Fig. 2A) or 300 s (Fig. 2B), while n was equal to 50 and p was equal to 0.3. In the simulations presented in Fig. 3, conducted for $\tau = 300$ s and for the standard resting rate constant of ATP usage the

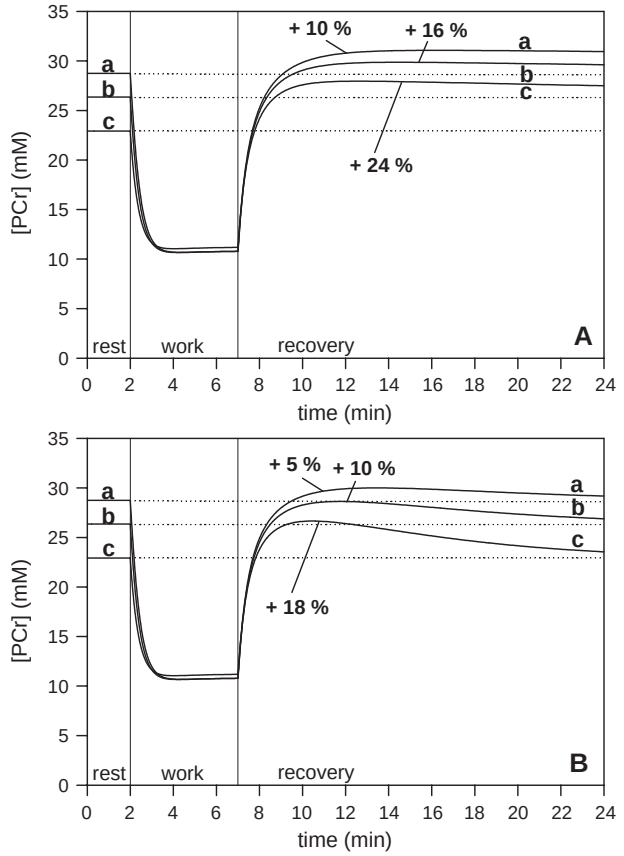

Fig. 2. Simulated effect of the resting PCr/Cr ratio on the relative PCr overshoot. Resting PCr/Cr was modulated by changing the rate constant of ATP usage in rest. Line a—standard rate constant, resting PCr/Cr=4.59; line b—standard rate constant increased twice, resting PCr/Cr=3.05; line c—standard rate constant increased four times, resting PCr/Cr=1.90. The values of the relative PCr overshoot are presented in each case. (A) Simulations for $\tau = 3000$ s; (B) simulations for $\tau = 300$ s.

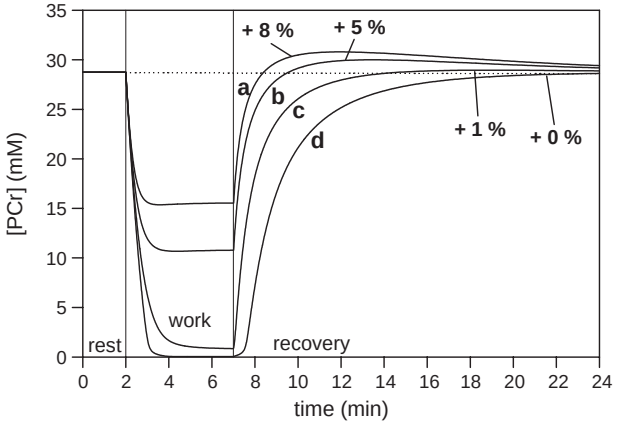

Fig. 3. Simulated effect of the extent of the parallel activation of oxidative phosphorylation during muscle work on the relative PCr overshoot. The extent of the parallel activation is determined by the power coefficient p. Line a—high parallel activation, $p = 0.4$; line b—medium parallel activation, $p = 0.3$; line c—low parallel activation, $p = 0.15$; line d—no parallel activation, $p = 0$. The values of the relative PCr overshoot are presented in each case. The simulations were performed for $\tau = 300$ s and for the standard resting ATP usage.

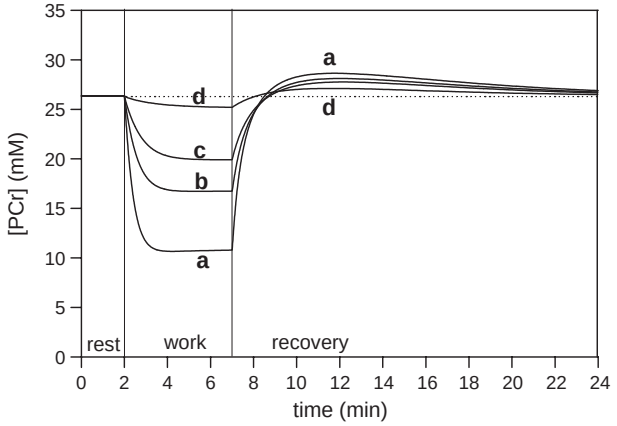

Fig. 4. Simulated effect of the work intensity on the relative PCr overshoot. The work intensity is determined by the relative rate constant of ATP usage during exercise. Line a—high work intensity; line b—medium work intensity; line c—low work intensity; line d—very low work intensity. The values of the relative PCr overshoot are presented in each case. The simulations were performed for $\tau = 300$ s and for the standard resting ATP usage multiplied by 2.

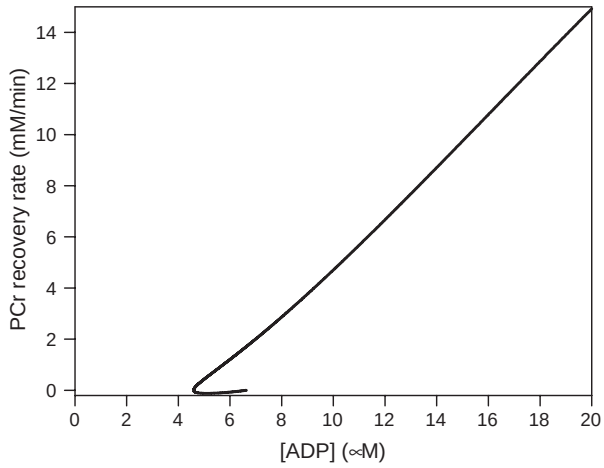

Fig. 5. Simulated relationship between PCr recovery rate and [ADP] in the case where ADP recovery undershoot takes place.

intensity of the parallel activation of oxidative phosphorylation was varied from no parallel activation ($p=0$) through low parallel activation ($p=0.15$) and medium parallel activation ($p=0.3$) to high parallel activation ($p=0.4$).

In the simulations presented in Fig. 4, performed for $\tau=300$ s and for a doubled resting rate constant of ATP usage the exercise intensity in subsequent simulations was varied by changing the relative increase in the rate constant of ATP usage at the onset of exercise n (see Eq. (1)) from 2.5 (line a) through 7.5 (line b) and 12.5 (line c) to 25 (line d). The simulation presented in Fig. 5 was performed for $\tau=3000$ s, $n=50$ and $p=0.3$. In all the above simulations the cytosolic pH was kept constant (pH=7.0). In the simulation shown in Fig. 6 the acidification of the cytosol by anaerobic glycolysis during very intensive exercise was involved. The activation of anaerobic glycolysis during exercise was adjusted to cause a decrease of pH to about 6.5 at the end of exercise. In order to cause a slow return of cytosolic pH to its resting value during muscle recovery the rate constant of the proton efflux from the muscle cell was decreased three times in relation to the 'standard' value of this constant. τ was equal to 300 s, the resting rate constant of ATP usage was twice greater than the standard resting rate constant of ATP usage and this constant was increased 25 times at the onset of exercise.

3. Theoretical results

As it was already mentioned above, the PCr recovery overshoot (as well as P_i undershoot and ADP undershoot) appears within the model of oxidative phosphorylation in skeletal muscle used in the present study when the characteristic decay time τ of the activation of oxidative phosphorylation is long enough. This is illustrated by the computer simulations presented in Fig. 1 that presents the changes over time of [PCr] in rest, during work and during recovery. For all the simulations the time courses of [PCr] are identical in rest and during exercise, and they differ during recovery depending on the decay time τ. One can easily see that for small values of τ (0 s, 10 s, 30 s) there is an asymmetry between PCr on- and off-transient kinetics—[PCr] decreases more quickly after the onset of exercise than increases after the termination of exercise. At medium values of τ (100 s) the PCr on-/off-transient kinetics is more or less symmetrical. At high values of τ (300 s, 1000 s, 3000 s) the PCr concentration rises above its resting level during muscle recovery—the PCr overshoot appears. In the last case, the time of duration of the overshoot strictly depends on the characteristic decay time of the direct activation of oxidative phosphorylation—this phenomenon may last from a few minutes to (potentially) several hours or even more. Therefore, the value of τ is crucial for the PCr off-kinetics, in particular for the phenomenon of the phosphocreatine recovery overshoot. Within our model the slow decay (long τ) of the direct activation of oxidative phosphorylation during recovery is absolutely necessary for the phenomenon

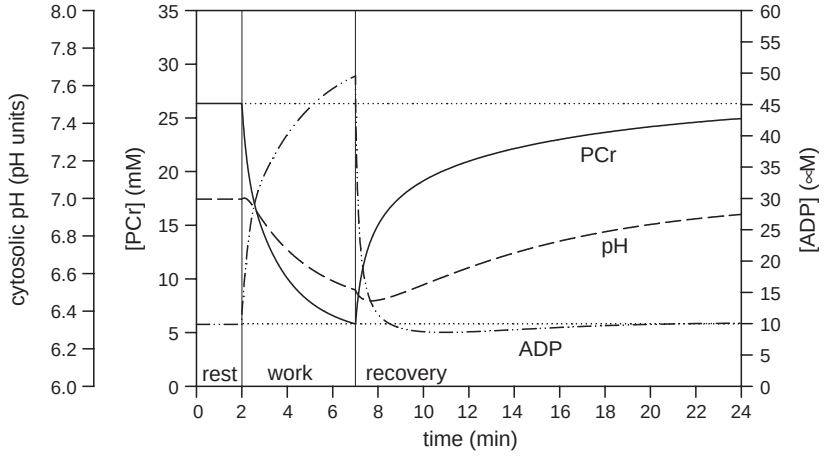

Fig. 6. Simulated time courses of [PCr], [ADP] and pH during rest, work and recovery for varying pH at high exercise intensity and lowered proton efflux intensity. The simulations were performed for $\tau=300$ s, for the standard resting ATP usage increased twice and for the proton efflux intensity divided by 3.

of PCr overshoot to appear—none of the other factors analyzed below is able to generate this overshoot if the direct activation of oxidative phosphorylation is turned off immediately after the termination of exercise.

Fig. 2 presents the simulated effect of the resting PCr/Cr ratio on the intensity of the PCr recovery overshoot for two values of τ: 3000 s (Fig. 2A) and 300 s (Fig. 2B). In the standard version of the model of oxidative phosphorylation in intact skeletal muscle used for computer simulations in the present study the resting PCr/Cr ratio is relatively high—it exceeds 4. This is mostly because a relatively low resting ATP usage and saturated oxygen concentration is assumed. However, this ratio can be significantly lowered by several factors, for instance a lowered (but still physiological) oxygen concentration, increased proton leak, increased resting ATP consumption or decreased amount of mitochondria. In order to test the impact of the resting PCr/Cr ratio on the size of the relative (expressed in % of the resting [PCr]) PCr overshoot we changed this ratio in computer simulations by changing the rate constant of ATP usage in rest, as explained in the Theoretical procedures (changes in oxygen concentration in the physiological range gave a qualitatively similar, but smaller effect). One can easily see that a decrease in the resting PCr/Cr ratio significantly increases the relative PCr overshoot. There are two reasons of this effect: (1) at low PCr/Cr the absolute PCr concentration (the reference point for the PCr overshoot) is relatively low; (2) at high PCr/Cr the resting concentration of Cr is low and it cannot be decreased much further during recovery, especially that in this case a relatively small decrease in [Cr] is equivalent to a relatively high increase in the ATP/ADP ratio and phosphorylation potential, while at low PCr/Cr [Cr] may decrease significantly without affecting very significantly the phosphorylation potential. The discussed theoretical predictions have been confirmed by our experimental results [Zoladz et al., unpublished data] showing a negative correlation between the resting PCr concentration and the relative extent of the PCr recovery overshoot.

Fig. 3 presents the simulated impact of the intensity of the parallel activation of oxidative phosphorylation on the PCr recovery overshoot. As it was discussed before, the intensity of the parallel activation is expressed in our model by the power coefficient p that determines the extent of the direct activation of oxidative phosphorylation (n^p) in parallel with an n-fold activation of ATP usage during rest-to-work transition. It can be seen in Fig. 3 that the PCr overshoot increases with an increase in the intensity of parallel activation. In the absence of parallel activation the PCr overshoot vanishes completely—this seems to be obvious, because PCr overshoot is caused in the simulations by a slow decay of the direct activation of oxidative phosphorylation during recovery. The delay in the PCr resynthesis at the beginning of recovery for no parallel activation (line d) results from the fact that in this case (high increase in ATP consumption, no direct activation of ATP production) most of ATP is converted during exercise into ADP and further into AMP (the reaction catalyzed by adenylate kinase), and it takes some time to re-convert AMP back to ADP and ATP during recovery.

Fig. 4 shows the simulated effect of the muscle work intensity on the extent of the PCr overshoot at a fixed intensity of parallel activation ($p=0.3$). As already mentioned previously (see Eq. (1) and the last paragraph) the work intensity is expressed within the model by the parameter n determining how many times the rate constant of ATP usage increases during rest-to-work transition. It can be clearly seen in Fig. 4 that the extent of the relative PCr overshoot increases with the work intensity. This effect can be explained by the fact that an increase in the activation of ATP usage during work in relation to rest (parameter n) increases also the extent of the direct activation of oxidative phosphorylation (n^p).

Because we have at disposal only a simplified semi-quantitative kinetic description of the proton production by anaerobic glycolysis [18], the above-presented simulations were performed for a constant cytosolic pH. However, when changes in pH during muscle work and recovery were taken into account in computer simulations, the general results concerning the PCr recovery overshoot were rather similar, although a slow return of pH to its resting value during recovery tended to delay and/or decrease the PCr overshoot (see below).

Within the model, PCr overshoot is accompanied by P_i undershoot, ADP undershoot and, in a consequence, phosphorylation potential overshoot. The ADP undershoot, observed in some experimental studies [3,9–11], leads to a dependence of the PCr recovery rate (corresponding to ATP synthesis rate minus ATP usage rate) on ADP concentration [11] where two different ATP synthesis rates may correspond to one ADP concentration. In other words, there is no unique ATP synthesis rate–[ADP] relationship, because the ATP synthesis rate depends not only on [ADP] (and, to a smaller extent, [P_i]), but also on the activity of oxidative phosphorylation that decreases in time during recovery. A simulated relationship between the PCr recovery rate and [ADP] (for $\tau=3000$ s) is presented in Fig. 5. It is quite similar to that obtained experimentally by Kemp et al. [11]; some differences may be partly due to the fact that a constant pH was maintained in the discussed computer simulation.

It is worth to mention that in several experimental studies [9–11] ADP undershoot is not accompanied by PCr overshoot. This finding can be explained by the fact that in those studies pH was low at the end of exercise and returned very slowly to its resting level during recovery; low pH shifted the CK equilibrium in the direction of a lowered PCr/Cr ratio at a given [ADP] and thus prevented the appearance of the PCr overshoot. In order to illustrate the discussed effect of a slow return during muscle recovery of the cytosolic pH from a low value at the end of exercise to the resting value, computer simulations using the version of

the model involving a simple kinetic description of proton production by anaerobic glycolysis [18] were performed. These simulations, performed for $\tau = 300$ s, are presented in Fig. 6. In order to slow down the return of pH to the resting value during recovery the standard value of the rate constant of proton efflux from the muscle cell was decreased three times. Fig. 6 clearly demonstrates that if the rate of the return of the cytosolic pH to the resting value is low (for instance as a result of a slow proton efflux from the muscle cell), the ADP undershoot may appear in the absence of PCr overshoot. If the standard rate constant of the proton efflux or a greater value of the decay time τ (3000 s) is used in computer simulations the PCr overshoot does not disappear completely, but is delayed and/or decreased (when pH decreases significantly during muscle work as a result of proton production by anaerobic glycolysis).

4. Discussion

The computer simulations conducted in the present study suggest that several factors/parameters may affect the appearance, duration and extent of the PCr recovery overshoot in skeletal muscle. These factors/parameters comprise the characteristic time τ of the decay during recovery of the direct activation of oxidative phosphorylation, resting PCr/Cr ratio (determined by resting ATP usage, mitochondria content, oxygen concentration and other factors), extent of the parallel activation of oxidative phosphorylation (parameter p), muscle work intensity (parameter n) as well as the cytosolic pH at the end of exercise and the rate of its return to the resting value during recovery. The question remains how these theoretical predictions refer to the experimental data available in the literature.

First of all the present theoretical studies can easily explain why the PCr overshoot is present in some cases and absent in other cases. As can be seen in Fig. 1 the PCr overshoot appears if the decay time τ of the activation of oxidative phosphorylation is long enough. Additionally, the longer the decay time, the longer the duration of the overshoot. In some cases the PCr level returns quite quickly to its resting value during recovery [1], especially if it is taken into account that this experiment was performed in 30 °C, while in other cases the PCr overshoot lasts over 1 h [6].

Second, the PCr overshoot tends to be higher if the resting PCr/Cr ratio is lower (Fig. 2). In some cases the PCr/Cr ratio is lower in oxidative (type I) muscle fibers [19], while in other cases it is lower in glycolytic (type II) muscle fibers [20]. This may be one of the reasons why this overshoot is present only in oxidative [2] or in glycolytic [5,7] muscle fibers. The resting PCr/Cr ratio may be different in different muscles/muscle fibers because of different resting ATP usage, mitochondria content, oxygen concentration, and/or proton leak intensity. Low PCr/Cr may in some cases cause the appearance of the PCr overshoot (if τ is not great enough to generate this overshoot at high PCr/Cr) and in other cases it can increase the size of PCr overshoot. However, it must be emphasized that low resting PCr/Cr cannot alone, without the slow decay of the activation of oxidative phosphorylation during recovery, generate the phosphocreatine recovery overshoot.

Third, the extent of the PCr overshoot is proportional to the intensity of parallel activation (p) (Fig. 3). As it was suggested previously [12] it is probable that this intensity is greater in oxidative muscle than in glycolytic muscle. This may be another explanation of the preferable appearance, at least in some cases, of the PCr overshoot in oxidative muscle/muscle fibers [1,2]. It was proposed that the intensity of the parallel activation increases as a result of muscle training [15]. This could suggest that the PCr overshoot is more likely to appear or has a greater extent in trained muscle than in untrained muscle (provided that other factors/parameters are unchanged), at least after a prolonged exercise where anaerobic glycolysis does not play an important role (see below).

Fourth, the extent of the PCr overshoot increases with an increase in work intensity/energy demand (n) (Fig. 4). To our knowledge, the discussed phenomenon is never observed at low work intensities—it appears either after a high-intensity short-term exercise where anaerobic glycolysis produces a substantial amount of ATP and protons [5,7] or after a quite intensive oxidative exercise where a significant decrease in [PCr] during work takes place [1–3]. In [1] the size of the PCr overshoot in oxidative muscle clearly depends on the work intensity and is completely absent at low work intensity. The last fact may suggest that also other factors/parameters, for instant the decay time τ, are greater/more intensive at high work intensity. It has been also shown [9] that the ADP undershoot (see below) appears at high, but not at low work intensity.

Fifth, if cytosolic pH decreases significantly during muscle exercise and then slowly returns to the resting value during recovery the PCr overshoot may be delayed/decreased or even vanish at all, if τ is not very long (Fig. 6). This may be the reason why this overshoot is absent even when ADP undershoot is present [9–11]. In the cited experimental studies the cytosolic pH returned to the resting level much more slowly than [PCr], what seems to confirm our theoretical predictions.

Of the above factors/parameters, the low resting PCr/Cr (at least in some cases), high intensity of parallel activation and lack of the slow pH recovery promote the appearance of the PCr overshoot in oxidative muscles/muscle fibers vs. glycolytic muscles/muscle fibers. This may explain why in some cases this overshoot is seen only in the former ones [1,2]. However, in some other cases the discussed phenomenon is also observed in glycolytic muscles/muscle fibers after a very intensive short-term exercise [5,7]. It was reported that the resting PCr/Cr ratio is sometimes greater in type I muscle fibers than in type II muscle fibers [20], but the difference was very small. Therefore, it is likely that in glycolytic muscle after very intensive exercise some other

factors may be most important for the appearance of the PCr overshoot. First, during very intensive exercise the increase in energy demand/ATP usage at the onset of exercise in relation to rest (parameter n) is very high (although a part of the energy demand is met by ATP production by anaerobic glycolysis). Second, in those conditions several stressing factors may appear, including acidification of the cytosol, low oxygen concentration (hypoxia), free radical production and mechanical stress. Such factors may lengthen the characteristic decay time τ of the direct activation of oxidative phosphorylation and thus promote the appearance of the PCr recovery overshoot and ADP undershoot. It has been observed that the extent of the ADP undershoot is proportional to the acidification of myocytes [10], what agrees well with our supposition. In our experimental studies [Zoladz et al., unpublished data] we observed a negative correlation between the muscle pH at the termination of short-term high-intensity exercise and the extent of the PCr recovery overshoot.

Low pH could potentially exert two opposite effects on the PCr overshoot: a slow return of pH to the resting value would delay/decrease this overshoot (see above), while the stress caused by acidification would enhance it. Additionally, the duration of the PCr overshoot seems to be longer in glycolytic muscles/muscle fibers than in oxidative muscles/muscle fibers, what further supports the above hypothesis. Generally, different factors/parameters may be most important for the appearance and size of the PCr overshoot in oxidative muscles/muscle fibers after intensive exercise and in glycolytic muscles/muscle fibers after very intensive short-term exercise, although in both cases this phenomenon can be explained by a slow decay of the direct activation of oxidative phosphorylation during muscle recovery.

The low oxygen concentration (hypoxia) in glycolytic muscle during very intensive exercise may become limiting for the ATP supply by oxidative phosphorylation (it may decrease the respiration rate because of substrate shortage). It is likely that in such conditions the intensity of parallel activation (parameter p) is increased in order to compensate this limitation and to increase the activity of oxidative phosphorylation. Such a mechanism might account for the appearance of/increase in the PCr overshoot in glycolytic skeletal muscle after very intensive exercise. It is also able to explain the PCr overshoot observed in heart after a period of ischemia [21,22].

In the present study when discussing the so-called glycolytic muscles we are referring to the mitochondria-rich type IIA muscle fibers, which, unlike IIX fibers that are rather poorly expressed in human locomotory muscles, exhibit quite a large activity of oxidative phosphorylation. The type IIA muscle fibers are called ‘glycolytic’ because anaerobic glycolysis is more active in them than in ‘oxidative’ muscles (containing predominantly type I muscle fibers) and they may produce substantial amounts of lactate. However, like in ‘oxidative’ muscles, the phosphocreatine resynthesis during recovery is purely oxidative in them, and therefore the mechanism underlying the phosphocreatine overshoot postulated by us is fully relevant also for ‘glycolytic’ muscles.

The question arises what can be the physiological role of the PCr recovery overshoot and the related P_i undershoot, ADP undershoot and phosphorylation potential overshoot. One of possible answers is muscle stress during exercise, caused for instance by a very low pH at the end of exercise, high mechanical work, free radical production and so on. Such stress could lead to damage of many proteins and other chemical compounds. Therefore, the need would appear to resynthesize quickly these components of the cell, and therefore to activate RNA synthesis, protein synthesis and other relevant processes. It has been demonstrated that RNA synthesis and protein synthesis are very sensitive (much more than e.g. sodium/potassium and calcium ion circulation) to the ATP/ADP ratio (and/or phosphorylation potential) [23]. Therefore, the function of the PCr overshoot and the related ADP undershoot could be to accelerate the repairing of the damages of muscle cells occurring during stressing exercise. In this context, the ADP undershoot would be the phenomenon that really matters ([ATP] is essentially constant in skeletal muscle under most conditions). As it was discussed above, in some cases the ADP undershoot is not accompanied by PCr overshoot. Even when both PCr overshoot and ADP undershoot are absent, an increase in τ can accelerate the decrease in [ADP] after the termination of exercise and therefore speed up the RNA and protein synthesis. The above general reasoning could explain the good correlation between the cellular pH at the end of exercise and the extent of the ADP undershoot encountered in [10].

Not only ADP undershoot, but also P_i undershoot may take place during muscle recovery in the presence [1,2] or absence [9] of PCr overshoot. Changes in $[P_i]$ are not a mirror image of changes in [PCr] during work and recovery in muscle, as could be expected on the basis of the Lohmann reaction alone, because some inorganic phosphate can be ‘trapped’ in the form of glycolysis intermediate metabolites (phosphate monoesters) [9,24]. The role of the P_i undershoot may be similar to the possible role of the ADP undershoot proposed before.

The question arises if the slow decay of the activation of oxidative phosphorylation is the only possible explanation of the PCr recovery overshoot. The only potential alternative mechanism we can imagine that could generate this overshoot is a lower ATP usage during recovery than in rest, at identical activity of oxidative phosphorylation during recovery and in rest. However, even if the ATP usage is completely switched off after the termination of exercise in computer simulations (which in reality would of course lead to the cell death, because such ATP-consuming reactions as ion circulation and protein synthesis would cease), the resultant PCr overshoot is small (3–4%) [simulations not shown].

The molecular factor/mechanism that directly activates all oxidative phosphorylation complexes during muscle work is still not identified experimentally. However, as it was discussed previously [12,14,25] this factor/mechanism seems to involve calcium ions and some hormones related to muscle metabolism (e.g. catecholamines, thyroid hormones, aldosteron). The parallel activation may consist e.g. in protein phosphorylation. In such a case, protein dephosphorylation would be responsible for a slow (exponential?) decay of the direct activation of oxidative phosphorylation during muscle recovery. The involvement of hormones in the parallel activation is confirmed by the fact that aldosteron may increase the extent of the PCr recovery overshoot [3].

In conclusion, in the present theoretical study we show that the duration and/or extent of PCr recovery overshoot in skeletal muscle is increased by a long characteristic decay time of the direct activation of oxidative phosphorylation, low resting PCr/Cr ratio, high intensity of the parallel activation of oxidative phosphorylation and high muscle work intensity, and it is delayed/decreased by a slow return of cytosolic pH to its resting value during muscle recovery. Therefore, the appearance, duration and size of PCr overshoot may be conditioned by several factors/parameters, the effects of which overlap. We suggest that differences in the above factors/parameters are most important for the genesis and size of the PCr overshoot in oxidative muscles/muscle fibers after intensive exercise and in glycolytic muscles/muscle fibers after very intensive short-term exercise. We postulate that the PCr overshoot, as well as the related ADP undershoot and phosphorylation potential overshoot are induced by muscle stress causing damage of proteins and other components of the cell. Finally, we hypothesize that the physiological role of the PCr overshoot or, in fact, the phosphorylation potential overshoot is to stimulate different processes, such as RNA synthesis and protein synthesis, that participate in the repairing of the damages of the muscle cells that occur during stressing exercise.

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