Regulation of oxidative phosphorylation in different muscles and various experimental conditions

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It has been shown previously that direct stimulation of oxidative-phosphorylation complexes in parallel with the stimulation of ATP usage is able to explain the stability of intermediate metabolite (ATP/ADP, phosphocreatine/creatine, NADH/NAD^+, protonmotive force) concentrations accompanied by a large increase in oxygen consumption and ATP turnover during transition from rest to intensive exercise in skeletal muscle. It has been also postulated that intensification of parallel activation in the ATP supply–demand system is one of the mechanisms of training-induced adaptation of oxidative phosphorylation in skeletal muscle. In the present paper, it is demonstrated, using the computer model of oxidative phosphorylation in intact skeletal muscle developed previously, that the direct activation of oxidative phosphorylation during muscle contraction can account for the following kinetic properties of oxidative phosphorylation in skeletal muscle encountered in different experimental studies: (i) increase in the respiration rate per mg of mitochondrial protein at a given ADP concentration as a result of muscle training and decrease in this parameter in hypothyroidism; (ii) asymmetry (different half-transition time, $t_{1/2}$) in phosphocreatine concentration time course between on-transient (rest → work transition) and off-transient (recovery after exercise); (iii) overshoot in phosphocreatine concentration during recovery after exercise; (iv) variability in the kinetic properties of oxidative phosphorylation in different kinds of muscle under different experimental conditions. No other postulated mechanism is able to explain all these phenomena at the same time and therefore the present paper strongly supports the idea of the parallel activation of ATP usage and different oxidative-phosphorylation complexes during muscle contraction.

Key words: mitochondrial respiration, oxidative phosphorylation, phosphocreatine, regulation of metabolism, skeletal muscle.

INTRODUCTION

Oxidative phosphorylation is the main source of energy, in the form of ATP, during active steady state in most types of muscle fibres at moderate and intensive (but not maximal) exercise. During transition from rest to work, the increased ATP demand must be matched by an elevated ATP supply in order to prevent fast complete exhaustion of ATP, which would lead to termination of exercise and possibly to muscle-cell death.

Three main mechanisms of adjusting the rate of ATP supply by oxidative phosphorylation to the current energy demand have been proposed in the literature. In the first mechanism, which will be called here the output-activation mechanism, only ATP usage (output of the system) is directly activated by Ca^{2+} ions during muscle work, whereas oxidative phosphorylation is activated only indirectly, through negative feedback involving an increase in [ADP] (and [P_i]). It was originally postulated by Chance and Williams [1,2] that the dependence of the respiration rate ($\dot{V}O_2$) on [ADP] is hyperbolic (first-order at low [ADP]). Jeneson et al. [3] modified this proposal and postulated that the [ADP]-dependence of $\dot{V}O_2$ is much steeper, at least second-order. The output-activation mechanism is presented in Scheme 1(A).

The discovery of the activation *in vitro* of the 'key' TCA (tricarboxylic acid) cycle dehydrogenases (pyruvate dehydrogenase, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase) by Ca^{2+} ions prompted several authors to postulate that substrate dehydrogenation (input of the system) is activated in parallel with ATP usage (input/output-activation mechanism) [4,5]. However, in this proposal, oxidative phosphorylation is still activated only indirectly, via an increase in [ADP] and/or an increase in the NADH/NAD^+ ratio. The input/output-activation mechanism is presented in Scheme 1(B).

Theoretical studies by means of the well-tested computer model of oxidative phosphorylation in skeletal muscle mitochondria developed previously [6] led to the conclusion that only a direct activation by some cytosolic factor of (almost) all oxidative phosphorylation enzymes is able to account for large changes in $\dot{V}O_2$ and ATP turnover accompanied by only very moderate increase in [ADP] during the rest → work transition in skeletal muscle [7–9]. This mechanism, called the each-step-activation mechanism or direct-activation mechanism is presented in Scheme 1(C).

The direct-activation mechanism is not only able to account for the relative stability in [ADP] {and also in [P_i], PCr (phosphocreatine)/creatine, NADH/NAD^+ and Δp (protonmotive force)} during the rest → work transition, but can also explain the fact that the maximum oxygen consumption (recalculated for the amount of mitochondrial proteins) in intact skeletal muscle is 2–4 times greater than in isolated mitochondria, skinned fibres, muscle homogenate or intact muscle during recovery [10,11]. Additionally, it was demonstrated that only an increase in parallel activation can account for the increase in the relative slope of the phenomenological [ADP]-dependence of $\dot{V}O_2$ as a result of muscle training encountered in experimental studies [12].

Nevertheless, the physical nature of the cytosolic factor directly activating particular oxidative-phosphorylation complexes still remains uncertain. It has been postulated that this factor can be found in the frequency of calcium oscillations generated by cells in response to external stimuli (hormones and neural signals), and that this frequency can be integrated over time by some protein

Abbreviations used: cyt *c*, cytochrome *c*; Δp, protonmotive force; PCr, phosphocreatine; $t_{1/2}$, half-transition time; TCA, tricarboxylic acid.

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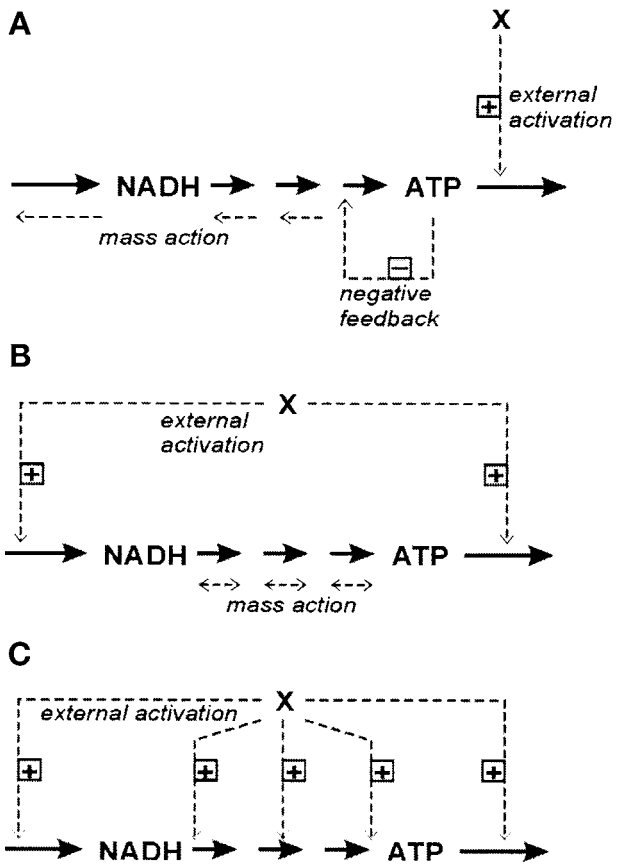

Scheme 1 Three possible mechanisms adjusting ATP supply to current energy demand

X, cytosolic factor(s) activating different components of the energetic system in muscle. (**A**) Output activation mechanism. X activates only ATP usage; (**B**) Input/output-activation mechanism. X activates ATP usage and substrate dehydrogenation; (**C**) Each-step activation. X activates ATP usage, substrate dehydrogenation and all oxidative phosphorylation complexes.

which causes, for example, phosphorylation of oxidative-phosphorylation complexes [8]. The parallel activation of Δp production and Δp consumption was directly demonstrated during stimulation of respiration by vasopressin in hepatocytes [13]. However, still more evidence is needed to prove ultimately that the each-step-activation mechanism actually takes place in skeletal muscle during the rest → work transition. On the other hand, the (potential) effect of this mechanism on various kinetic properties of oxidative phosphorylation is interesting. Finally, the question arises whether or not the quantitative extent of parallel activation is identical in different muscle types under different experimental conditions. If it is not, it would be interesting to test if differences in the extent of parallel activation are able to account for the differences in the kinetic properties of oxidative phosphorylation in different muscle types (glycolytic skeletal muscle, oxidative skeletal muscle, heart) studied under different conditions (perfused muscle compared with muscle *in situ*, electrically stimulated muscle compared with neurally stimulated muscle).

In the present computer-aided theoretical study it is shown that the idea of direct activation of oxidative phosphorylation implies or at least is able to account for several kinetic properties of oxidative phosphorylation in muscle that are encountered in experimental studies. Namely, it explains why $\dot{V}O_2$ per mg of mitochondrial protein at a given [ADP] is greater in trained muscle than in untrained muscle, and is less in hypothyroid muscle in relation to normal muscle [14]; it provides the reason for the PCr time-course asymmetry in the on- (rest → work transition after the onset of exercise) and off-transient (work → rest transition after exercise) [20–22]; it constitutes one of possible reasons for [PCr] overshoot above the resting level during muscle recovery after exercise [15]; finally, it suggests the reason of differences in the kinetic properties of oxidative phosphorylation in different muscles and experimental conditions [15–23].

THEORETICAL PROCEDURES

The previously developed computer model of oxidative phosphorylation in intact skeletal muscle [11] 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 TCA 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 and ATP usage. The time variations of the metabolite concentrations that constitute independent variables (NADH, ubiquinol, reduced form of cytochrome *c* (cyt *c*), O_2, internal protons, internal ATP, internal P_i, external ATP, external ADP, external P_i, external protons and 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.

In the simulations presented in Figures 1–4, ATP demand was increased 15 times (Figure 1) or 50 times (Figures 2–4) at the onset of exercise, in order to cause a decrease in [PCr] to approx. 11 mM, and was decreased back to the resting value during recovery after termination of exercise. The activities (rate constants) of oxidative-phosphorylation enzymes were not increased at the onset of exercise in the simulation presented in Figure 1 and were increased $50^{0.3}$ times in the simulations presented in Figures 2–4. After termination of exercise, these activities remained unchanged (Figure 1), were instantly decreased to the rest values (Figure 2) or decreased exponentially with time to the rest values (Figures 3 and 4). This exponential decay of activation of oxidative-phosphorylation complexes during recovery was described by the following expression:

$$m = 1 + (50^{0.3} - 1) \cdot e^{-t/\tau} \quad (1)$$

where m is the multiplicity of resting complex activity, t stands for time and τ is the characteristic time constant of the decay of activation of oxidative phosphorylation. The value of τ was equal to 30 s (Figure 3) or 5 min (Figure 4).

In all simulations presented in Figures 1–4, the cytosolic pH was kept constant in order to exclude the effect of changes in this parameter on the half-transition times, $t_{1/2}$, of PCr (and $\dot{V}O_2$) [24]. It has been confirmed that this procedure does not change the general conclusions drawn in the present article.

THEORETICAL RESULTS AND DISCUSSION

Activity of oxidative-phosphorylation enzymes

Parallel activation is equivalent to an increase in the activity of oxidative-phosphorylation enzymes during muscle work.

Therefore an increase in parallel activation should increase the respiration rate per mg of mitochondrial protein at a given [ADP]. Particular oxidative-phosphorylation enzymes are simply more active during exercise at some fixed ADP level in the case of high parallel activation than in the case of low (or no) parallel activation. This is, in fact, the most direct and evident kinetic effect of parallel activation on oxidative phosphorylation. The discussed property is illustrated in Figure 1 in [12].

Dudley and co-workers [14] studied the effect of hypothyroidism and training on the relation between [ADP] and $\dot{V}O_2$ in muscle. The authors observed that muscle training significantly increases the relative slope of the [ADP]-dependence of $\dot{V}O_2$. As discussed previously [12], the increase in the relative value of this slope corresponding to the phenomenological $\dot{V}O_2$ sensitivity to [ADP] defined as:

$$R = \frac{\dot{V}O_{2(work)}/\dot{V}O_{2(rest)}}{[ADP]_{(work)}/[ADP]_{(rest)}} \quad (2)$$

can be only explained by an increase in parallel activation from a low value in untrained muscle to a high value in trained muscle. Since hypothyroidism has the opposite effect to the effect of training {it significantly decreases the discussed sensitivity (at least at moderate work intensities) [14]} it is reasonable to assume that, in this case, parallel activation is decreased to very low values or is switched off. Both training and hypothyroidism change mitochondria/mitochondrial protein content in muscle significantly. Therefore Dudley et al. [14] recalculated oxygen uptake per cyt *c* concentration units. They observed that training of muscle increases $\dot{V}O_2$/cyt *c* at some given [ADP], whereas hypothyroidism decreases the value of this parameter. It is not certain if the cyt *c* content reflects exactly the overall mitochondrial protein content, because the content of different mitochondrial proteins may change to a different extent as a result of training or hypothyroidism. Additionally, at high work intensities, the picture is not as clear as at moderate work intensities [14]. Therefore the above results should be treated as being only semi-quantitative. However, the general effect clearly suggests that muscle training increases the direct stimulation of oxidative phosphorylation during muscle work (at least at moderate work intensities), and hypothyroidism decreases this stimulation. The last conclusion suggests an important role of hormones in the regulation of the ATP supply by oxidative phosphorylation in skeletal muscle.

Phenomenological sensitivity of oxidative phosphorylation to [ADP] or [PCr]

The differences in the phenomenological sensitivity of $\dot{V}O_2$ to [ADP] defined in eqn (2) between muscles of different training status testify to different extents of parallel activation in these muscles [12]. However, such differences in sensitivities also exist between oxidative (slow-twitch) muscle and glycolytic (fast-twitch) muscle studied under similar conditions (perfused muscle stimulated electrically). The $\{\dot{V}O_{2(work)}/\dot{V}O_{2(rest)}\}/\{[PCr]_{(work)}/[PCr]_{(rest)}\}$ ratio is three times greater in oxidative muscle (soleus) (ratio = 6:1) than in glycolytic muscle (biceps) (ratio = 2:1) [15]. Therefore the direct stimulation of oxidative phosphorylation seems to be much greater in type I muscle fibres than in type IIA muscle fibres and, especially, type IIB(X) muscle fibres. This conclusion is consistent with the intensification of parallel activation as a result of muscle training, since training causes transformation of type IIB(X) fibres into type IIA fibres, and further into type I muscle fibres.

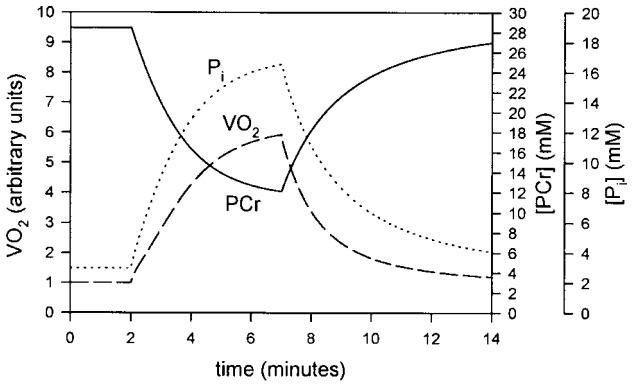

Figure 1 Simulated on- and off-transients of [PCr], [P_i] and $\dot{V}O_2$ in the absence of parallel activation

Rest, 0–2 min; exercise, 2–7 min; recovery, 7–14 min.

[PCr] $t_{1/2}$ during the on- and off-transient

Figure 1 presents simulated time courses of [PCr], [P_i] and $\dot{V}O_2$ during the on- and off-transient in the absence of parallel activation. At the onset of exercise, the rate constant of ATP usage was increased 15 times in order to cause a fall in [PCr] to 11 mM (in the active steady state, which is not completely achieved in the presented simulation during 5 min of exercise), and at the termination of exercise, the resting ATP demand was restored. The rate constants of oxidative-phosphorylation complexes were kept unchanged during the whole simulation.

One can see that an approx. 2.5-fold decrease in [PCr] (and an approx. 9-fold increase in [ADP]; results not shown) is accompanied by > 6-fold increase in $\dot{V}O_2$ (in this case, the [ADP]-dependence of $\dot{V}O_2$ follows a simple hyperbolic, Michaelis–Menten kinetics). The $t_{1/2}$ for both [PCr] and $\dot{V}O_2$ are relatively long, at > 1 min. Additionally, the [PCr] on-transient and off-transient are (almost) symmetrical; in particular, in both cases, $t_{1/2} = 78$ s.

The above pattern of kinetic behaviour of oxidative phosphorylation in muscle, namely (i) a small increase in the respiration rate in relation to decrease in [PCr] and increase in [ADP], (ii) long half-transition times, and (iii) symmetry of $t_{1/2}$ for PCr on- and off-transient kinetics, is observed in some experimental studies on glycolytic muscle in rather artificial (non-physiological) conditions: perfused muscle and/or electrically stimulated muscle [15–17]. Therefore it can be concluded that, under those conditions, the parallel activation in the ATP supply–demand system is very low or completely absent. This may be one of the reasons why several authors consider the negative feedback via [ADP] as the only regulatory mechanism responsible for adjusting the rate of ATP supply by oxidative phosphorylation to the current energy demand. It should be emphasized that the (at least) second-order mechanistic-dependence of oxidative phosphorylation on [ADP] postulated by Jeneson et al. [3] is not needed at all to explain the behaviour of the system encountered in the above-cited experiments.

Figure 2 presents the simulated changes over time of [PCr], [P_i] and $\dot{V}O_2$ in the case of a direct stimulation of particular oxidative-phosphorylation enzymes during muscle work. In this simulation, oxidative phosphorylation was activated at the onset of exercise $50^{0.3}$ times, in parallel with a 50-fold activation of ATP usage (medium-intensity exercise); the extent of this activation was selected in order to cause the same decrease in [PCr] (to approx. 11 mM) as in the simulation presented in Figure 1. At the

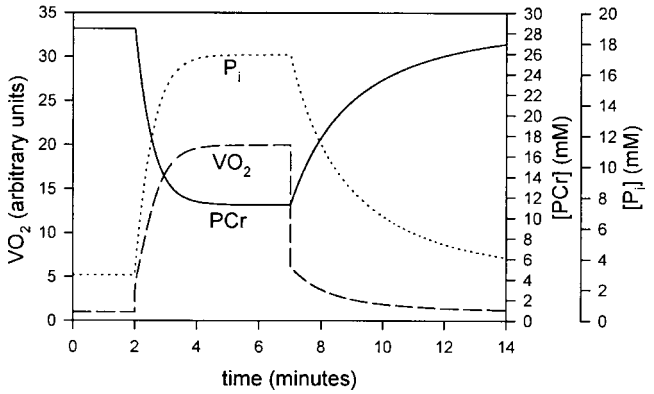

Figure 2 Simulated on- and off-transients of [PCr], [P_i] and $\dot{V}O_2$ in the presence of parallel activation

Activation of oxidative phosphorylation is switched on at the onset of exercise and switched off instantly at the termination of exercise. Rest, 0–2 min; exercise, 2–7 min; recovery, 7–14 min.

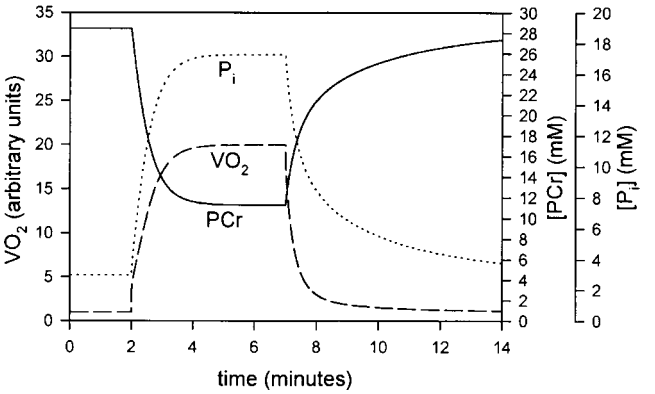

Figure 3 Simulated on- and off-transients of [PCr], [P_i] and $\dot{V}O_2$ in the presence of parallel activation

Activation of oxidative phosphorylation is switched on at the onset of exercise and decays exponentially after the termination of exercise with the time constant $\tau = 30$ s. Rest, 0–2 min; exercise, 2–7 min; recovery, 7–14 min.

termination of exercise and onset of recovery, the rate constants of both ATP usage and oxidative-phosphorylation complexes were lowered instantly to their rest values. It can be seen that parallel activation causes three main effects. First, a much greater (approx. 20-fold) increase in $\dot{V}O_2$ accompanies the same decrease in [PCr] (and increase in [ADP]); as a consequence, the 'order' of the phenomenological [ADP]-dependence of $\dot{V}O_2$ is here much greater than one. Secondly, the $t_{1/2}$ for [PCr] (and $\dot{V}O_2$) are much shorter. Thirdly, there appears to be an asymmetry in $t_{1/2}$ for PCr in the on- and off-transient. The instant switching off the activation of oxidative phosphorylation at the termination of exercise causes an essential discontinuity in the time course of oxygen consumption.

However, it is likely that the decay of activation of oxidative phosphorylation (consisting of, for example, dephosphorylation of oxidative-phosphorylation complexes) during muscle recovery is not instant, but follows some exponential characteristics with a characteristic time constant τ. Figure 3 presents a simulation that is strictly analogous to the simulation shown in Figure 2, but involves an exponential decay of the activation of oxidative phosphorylation following eqn (1) with τ equal to 30 s. It can be seen that the time course of $\dot{V}O_2$ becomes smooth now. Nevertheless, there is still asymmetry in $t_{1/2}$ for PCr: 24 s for on-transient and 39 s for off-transient. This difference is simply due to the fact that oxidative phosphorylation is more active during muscle work than during recovery. {However, it must be emphasized that the discussed asymmetry vanishes almost completely during low-intensity exercise, where [PCr] falls by $< 10\,\%$ of its resting value (results not shown), because under these conditions the direct activation of oxidative phosphorylation is very low.} Finally, $t_{1/2}$ for both PCr and $\dot{V}O_2$ is short now, shorter than 40 s.

The above simulations were performed for constant pH in order to exclude the possibility that changes in proton concentration are responsible for, for example, the asymmetry in [PCr] on- and off-transient kinetics. When changes in pH were allowed in computer simulations, the general picture did not change, although the values of $t_{1/2}$ were slightly different (theoretical results not shown; see also [24]).

The above set of simulated kinetic properties is characteristic of skeletal muscle (especially oxidative skeletal muscle) in physiological conditions: muscle *in situ* stimulated neurally [3,15,18–23]. First, in these experimental conditions, a large increase in $\dot{V}O_2$ during muscle work is accompanied by only very moderate changes in [PCr] and [ADP] [3,14,15,18]. Secondly, $t_{1/2}$ in on-transient for both PCr and $\dot{V}O_2$ is relatively short, ranging from 20 s to 50 s (depending on, for example, training status) [19–22]. [Nevertheless, one should bear in mind that the short $t_{1/2}$ may be partly due to high mitochondria content [12]; on the other hand, a difference as great as approx. 4-fold in the mitochondria content would be necessary to explain the difference in the transition time of 78 s compared with 24 s (theoretical results not shown)]. Thirdly, the asymmetry in [PCr] on- and off-transient kinetics appears: $t_{1/2}$ in off-transient is about 1.5 times greater than $t_{1/2}$ in on-transient [20–22]. This pattern of behaviour agrees well with the simulation presented in Figure 3. Also, the simulated changes, over time, in [P_i] reflect experimental studies well [21].

Therefore it can be concluded that in skeletal muscle (especially oxidative skeletal muscle) under physiological conditions (muscle *in situ* stimulated neurally), the direct activation of particular oxidative-phosphorylation enzymes constitutes the main regulatory mechanism responsible for adjusting the rate of ATP supply by oxidative phosphorylation to the current energy demand, whereas the negative feedback via [ADP] is only a minor, fine-tuning mechanism as postulated previously [9].

It must be emphasized that the pulmonary oxygen-uptake kinetics is characterized by a longer $t_{1/2}$ for $\dot{V}O_2$ in off-transient than in on-transient [20]. This property is not predicted by the computer model of oxidative phosphorylation used in the present study; in fact, the simulated $t_{1/2}$ for $\dot{V}O_2$ is even shorter in off-transient than in on-transient, in both the system without (Figure 1) and with (Figure 3) parallel activation. However, one should bear in mind that the model discussed does not involve many phenomena that could be responsible for lengthening $t_{1/2}$ for the pulmonary oxygen uptake in off-transient, for instance lactate utilization, slow component in oxygen-uptake kinetics or oxygen consumption by heart, respiratory muscles and other tissues. On the other hand, the simulated $t_{1/2}$ in on-transient for PCr and for $\dot{V}O_2$ are equal, in accordance with experimental results [20]. This fact may suggest that the pulmonary oxygen uptake reflects the oxygen consumption by muscle at the onset of exercise well.

[PCr] overshoot

It seems to be interesting what will happen if, in some cases, the characteristic time of the decay of activation of oxidative phosphorylation during recovery (τ) is much longer than 30 s.

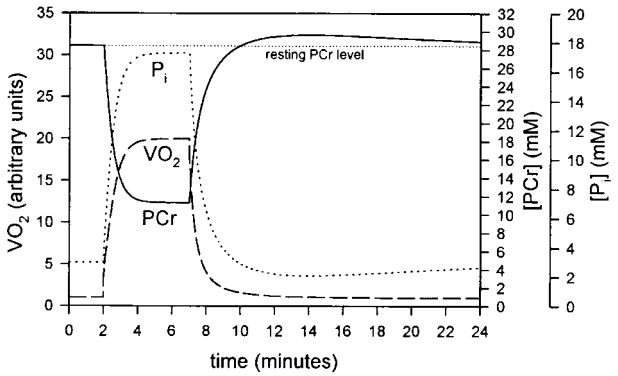

Figure 4 Simulated on- and off-transients of [PCr], [P_i] and $\dot{V}O_2$ in the presence of parallel activation

Activation of oxidative phosphorylation is switched on at the onset of exercise and decays exponentially after the termination of exercise with the time constant $\tau = 5$ min. Rest, 0–2 min; exercise, 2–7 min; recovery, 7–14 min.

Figure 4 presents a simulation analogous to the simulation shown in Figure 3, but with τ equal to 300 s. The most striking difference is that now the [PCr] rises during recovery even above its resting value, and then slowly approaches the resting level.

Such a behaviour is sometimes observed in experimental studies [15,25], but no convincing explanation of this phenomenon has been proposed so far. One possible explanation, resulting from the simulation presented in Figure 4, is that, in some cases, the direct stimulation of oxidative phosphorylation is not switched off instantly or quickly after the termination of exercise, but it still lasts for several minutes during muscle recovery.

General discussion

The idea of parallel activation (direct stimulation of oxidative phosphorylation and ATP usage during muscle contraction) was originally founded on the basis of the fact that, in intact skeletal muscle under physiological conditions, a large decrease in the respiration rate and ATP turnover is accompanied by only moderate changes in [ADP] during muscle contraction [7–9]. The computer model of oxidative phosphorylation in muscle mitochondria tested for a broad range of parameters and system properties predicted that neither the original output-activation mechanism nor the input/output-activation mechanism is able to account for this kinetic property of oxidative phosphorylation in skeletal muscle [7]. Jeneson et al. [3] proposed a modification of the output-activation mechanism with a more than second-order mechanistic-dependence of oxidative phosphorylation on [ADP]. This proposal can potentially explain the steep slope of the [ADP]-dependence of $\dot{V}O_2$ encountered in several experimental studies. However, it contradicts the kinetic properties of oxidative phosphorylation in isolated mitochondria and skinned fibres as well as the fact that NADH/NAD^+ and Δp are relatively stable and can even increase during the on-transient [8,26]. Additionally, Jeneson et al. [3] do not distinguish between relative changes in oxygen consumption and ATP turnover; the latter are over twice greater than the former due to proton leak, as discussed in [11]. Furthermore, the relative increase in ATP turnover estimated by Jeneson et al. [3] from the initial slope of PCr decomposition at the onset of exercise may be essentially underestimated if the parallel activation takes place (in such a case, the initial slope would be only a measure of the difference between increased ATP usage and activated ATP supply by mitochondria, and not of ATP usage alone). Finally, an infinite order of the mechanistic [ADP]-dependence of $\dot{V}O_2$ would be necessary in order to explain the fact that a 5-fold increase in $\dot{V}O_2$ in intact heart *in situ* is accompanied by essentially no changes in [ADP], [PCr] and [P_i] [27,28].

The 'phenomenological order' of the [ADP]-dependence of $\dot{V}O_2$ changes as a result of muscle training, as discussed previously [12]. This fact cannot be reconciled with any constant (first, second or greater) mechanistic [ADP]-dependence of $\dot{V}O_2$ order. Additionally, the parallel activation of Δp supply and Δp demand has been directly demonstrated during activation by vasopressin of oxidative phosphorylation in hepatocytes [13]. It has been also demonstrated that Ca^{2+} ions directly stimulate to a similar extent both Δp production and Δp consumption in isolated mitochondria of heart [29] and skeletal muscle [30].

The present paper aimed to support further the idea of parallel activation, and at the same time to investigate the kinetic effects of this mechanism on oxidative phosphorylation in skeletal muscle. These two tasks have appeared to be convergent, because the direct stimulation of oxidative phosphorylation during muscle contraction is able to account for several phenomena, which have been not yet explained and cannot be explained by the postulated greater than second-order mechanistic-dependence of $\dot{V}O_2$ on [ADP] [3].

First, only variations in the direct activation of oxidative phosphorylation complexes can explain the fact that the respiration rate per mg of mitochondrial protein at the same [ADP] is greater in trained muscle than in untrained muscle, and in untrained muscle than in hypothyroid muscle. Even a steep mechanistic [ADP]-dependence of $\dot{V}O_2$ implies a unique relationship between [ADP] and respiration rate per mg of mitochondrial protein.

Secondly, the presence of parallel activation leads to the asymmetry in PCr on- and off-transient kinetics. This asymmetry is due to the fact that oxidative-phosphorylation complexes are more active during muscle work than during recovery after exercise. Because the activity of oxidative phosphorylation is assumed to be constant within the output-activation mechanism, the asymmetry cannot appear in this case.

There is a theoretical possibility that glycolytic ATP supply can be, at least partially, responsible for the discussed on/off PCr asymmetry. However, under the conditions considered (moderate-to-intensive, but not maximal short-term exercise), oxidative phosphorylation is the main source of ATP. Preliminary computer simulations suggest that the presence of a glycolytic ATP supply tends even to diminish the discussed asymmetry (results not shown).

Thirdly, if it is assumed that, in some cases, the activation of oxidative phosphorylation decays slowly during recovery, the [PCr] overshoot appears. It is not clear if this assumption is correct or if there are other explanations of this phenomenon. Nevertheless, the idea of parallel activation seems to offer here at least a possible explanation; this is not the case for the output-activation mechanism.

Fourthly, parallel activation can account for differences in kinetic properties of oxidative phosphorylation in different muscle types and experimental conditions. Oxidative muscle, muscle *in situ* and muscle stimulated neurally tend to have a steep phenomenological [ADP]-dependence of $\dot{V}O_2$, short $t_{1/2}$ for [PCr] and $\dot{V}O_2$, as well as an asymmetry between on- and off-transient [PCr] kinetics, whereas glycolytic muscle, perfused muscle and electrically stimulated muscle are characterized by a less steep phenomenological [ADP]-dependence of $\dot{V}O_2$, longer $t_{1/2}$ and symmetrical on- and off-transients of [PCr]. These differences should not take place in the case of the output-activation mechanism; on the other hand, they can be easily explained by differences in the degree of direct stimulation of oxidative-phosphorylation complexes.

A similar situation takes place in the case of the kinetic properties of oxidative phosphorylation in heart. First of all, no changes in intermediate metabolite concentrations ([ADP], [PCr] and [P_i]) take place during 5-fold changes in work intensity and oxygen consumption in intact heart *in situ* [27,28], suggesting a perfect balance of activation of ATP supply and ATP usage, even better than in well-trained oxidative muscle under physiological conditions. Secondly, in perfused heart, the changes in [ADP] and phosphorylation potential between different workloads are generally greater than in heart *in situ* and depend on the respiratory substrates and hormones present in the perfusion medium [31]. Therefore, as in muscle, the parallel activation in heart seems to be very sensitive to different experimental conditions.

Why is the parallel activation greater in oxidative muscle, in muscle *in situ* and/or in neurally stimulated muscle than in glycolytic muscle, in perfused muscle and/or in electrically stimulated muscle? As oxidative muscle compared with glycolytic muscle the problem can be reversed, it can be said that oxidative muscle is muscle that has not only more mitochondria, but, first of all, a more intensive parallel activation than glycolytic muscle that greatly increases the oxidative ATP supply capacity of oxidative muscle, and therefore the latter does not have to rely on glycolytic ATP production. In the perfused muscle system, some factors, such as different hormones, that are important for intracellular signalling, and thus for parallel activation, may be lacking in the perfusion medium. Finally, electrical stimulation of muscle may disturb a subtle balance and circulation of ions, e.g. Ca^{2+} ions, across the cellular membrane, sarcoplasmic membrane and/or inner mitochondrial membrane.

CONCLUSIONS

The present paper, investigating the kinetic effect of parallel activation of ATP supply and ATP demand on oxidative phosphorylation in skeletal muscle, decidedly supports the idea of direct stimulation of particular oxidative-phosphorylation complexes and ATP usage during muscle contraction. This mechanism is able to explain the training-induced increase and hypothyroidism-induced decrease in the activity of oxidative-phosphorylation enzymes at a given [ADP], the asymmetry in [PCr] on- and off-transient kinetics, the [PCr] overshoot during muscle recovery, and the variability of the kinetic properties of oxidative phosphorylation in different muscle types and various experimental conditions. No other known mechanism is able to account for all of these phenomena and for several other phenomena discussed in previous articles. Thus the idea of parallel activation helps to interpret and systematize the great amount of experimental data available in the literature.

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REFERENCES

1 Chance, B. and Williams, G. R. (1955) Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. J. Biol. Chem. **217**, 383–393
2 Chance, B. and Williams, G. R. (1956) The respiratory chain and oxidative phosphorylation. Adv. Enzymol. **17**, 65–134
3 Jeneson, J. A., Wiseman, R. W., Westerhoff, H. V. and Kushmerick, M. J. (1996) The signal transduction function of oxidative phosphorylation is at least second order in ADP. J. Biol. Chem. **271**, 27995–27998
4 McCormack, J. G., Halestrap, A. P. and Denton, R. M. (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol. Rev. **70**, 391–425
5 Hansford, R. G. (1980) Control of mitochondrial substrate oxidation. Curr. Top. Bioenerg. **10**, 217–277
6 Korzeniewski, B. and Mazat, J.-P. (1996) Theoretical studies on the control of oxidative phosphorylation in muscle mitochondria: application to mitochondrial deficiencies. Biochem. J. **319**, 143–148
7 Korzeniewski, B. (1998) Regulation of ATP supply during muscle contraction: theoretical studies. Biochem. J. **330**, 1189–1195
8 Korzeniewski, B. (2000) Regulation of ATP supply in mammalian skeletal muscle during resting state → intensive work transition. Biophys. Chem. **83**, 19–34
9 Korzeniewski, B. (2001) Theoretical studies on the regulation of oxidative phosphorylation in intact tissues. Biochim. Biophys. Acta **1504**, 31–45
10 Tonkonogi, M. and Sahlin, K. (1997) Rate of oxidative phosphorylation in isolated mitochondria from human skeletal muscle: effect of training status. Acta Physiol. Scand. **161**, 345–353
11 Korzeniewski, B. and Zoladz, J. A. (2001) A model of oxidative phosphorylation in mammalian skeletal muscle. Biophys. Chem. **92**, 17–34
12 Korzeniewski, B. and Zoladz, J. A. (2003) Training-induced adaptation of oxidative phosphorylation in skeletal muscle. Biochem. J. **374**, 37–40
13 Korzeniewski, B., Harper, M.-E. and Brand, M. D. (1995) Proportional activation coefficients during stimulation of oxidative phosphorylation by lactate and pyruvate or by vasopressin. Biochim. Biophys. Acta **1229**, 315–322
14 Dudley, G. A., Tullson, P. C. and Terjung, R. L. (1987) Influence of mitochondrial content on the sensitivity of respiratory control. J. Biol. Chem. **262**, 9109–9114
15 Meyer, R. A. and Foley, J. M. (1994) Testing models of respiratory control in skeletal muscle. Med. Sci. Sports Exercise **26**, 52–57
16 Meyer, R. A. (1988) A linear model of muscle respiration explains monoexponential phosphocreatine changes. Am. J. Physiol. **254**, C548–C553
17 Kushmerick, M. J., Meyer, R. A. and Brown, T. R. (1992) Regulation of oxygen consumption in fast- and slow-twitch muscle. Am. J. Physiol. **263**, C598–C606
18 Hochachka, P. (1994) Muscles as metabolic machines. CRC Press, Boca Raton
19 Hickson, R. C., Bomze, H. A. and Holloszy, J. O. (1978) Faster adjustment of O_2 uptake to the energy requirement of exercise in the trained state. J. Appl. Physiol. **44**, 877–881
20 Rossiter, H. B., Ward, S. A., Kowalchuk, J. M., Howe, F. A., Griffiths, J. R. and Whipp, B. J. (2002) Dynamic asymmetry of phosphocreatine concentration and O_2 uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. J. Physiol. **541**, 991–1002
21 Yoshida, T and Watari, H. (1993) ^{31}P-Nuclear magnetic resonance spectroscopy study of the time course of energetic metabolism during exercise and recovery. Eur. J. Appl. Physiol. **66**, 494–499
22 Kushmerick, M. J. and Meyer, R. A. (1985) Chemical changes in rat leg muscle by phosphorus nuclear magnetic resonance. Am. J. Physiol. **248**, C542–C549
23 Constable, S. H., Favier, R. J., McLane, J. A., Fell, R. D., Chen, M. and Holloszy, J. O. (1987) Energy metabolism in contracting rat skeletal muscle: adaptation to exercise training. Am. J. Physiol. **253**, C316–C322
24 Korzeniewski, B. and Zoladz, J. A. (2002) Influence of rapid changes in cytosolic pH on oxidative phosphorylation in skeletal muscle: theoretical studies. Biochem. J. **365**, 249–258
25 Sahlin, K., Soderlund, K., Tonkonogi, M. and Hirakoba, K. (1997) Phosphocreatine content in single fibres of human muscle after sustained submaximal exercise. Am. J. Physiol. **273**, C172–C178
26 Rolfe, D. F. S., Newman, J. M. B., Buckingham, J. A., Clark, M. G. and Brand, M. D. (1999) Contribution of mitochondrial proton leak to respiration rate in working skeletal muscle and liver to SMR. Am. J. Physiol. **276**, C692–C699
27 Balaban, R. S., Kantor, H. L., Katz, L. A. and Briggs, R. W. (1986) Relation between work and phosphate metabolite in the *in vivo* paced mammalian heart. Science **232**, 1121–1123
28 Katz, L. A., Swain, J. A., Portman, M. A. and Balaban, R. S. (1989) Relation between phosphate metabolites and oxygen consumption of heart *in vivo*. Am. J. Physiol. **256**, H265–H274
29 Mildaziene, V., Baniene, R., Nauciene, Z., Marcinkeviciute, A., Morkuniene, M., Borutaite, V., Kholodenko, B. N. and Brown, G. C. (1996) Ca^{2+} stimulates both the respiratory and phosphorylation subsystems in rat heart mitochondria. Biochem. J. **320**, 329–334
30 Kavanagh, N. I., Ainscow, E. K. and Brand, M. D. (2000) Calcium regulation of oxidative phosphorylation in rat skeletal muscle mitochondria. Biochim. Biophys. Acta **1457**, 57–70
31 From, A. H. L., Zimmer, S. D., Michurski, S. P., Mohanakrishnan, P., Ulstad, V. K., Thoma, W. J. and Ugurbil, K. (1990) Regulation of oxidative phosphorylation rate in the intact cell. Biochemistry **29**, 3731–3743

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