Influence of rapid changes in cytosolic pH on oxidative phosphorylation in skeletal muscle: theoretical studies

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Cytosolic pH in skeletal muscle may vary significantly because of proton production/consumption by creatine kinase and/or proton production by anaerobic glycolysis. A computer model of oxidative phosphorylation in intact skeletal muscle developed previously was used to study the kinetic effect of these variations on the oxidative phosphorylation system. Two kinds of influence were analysed: (i) via the change in pH across the inner mitochondrial membrane and (ii) via the shift in the equilibrium of the creatine kinase-catalysed reaction. Our simulations suggest that cytosolic pH has essentially no impact on the steady-state fluxes and most metabolite concentrations. On the other hand, rapid acidification/alkalization of cytosol causes a transient decrease/increase in the respiration rate. Furthermore, changes in pH seem to affect significantly the kinetic properties of

transition between resting state and active state. An increase in pH brought about by proton consumption by creatine kinase at the onset of exercise lengthens the transition time. At intensive exercise levels this pH increase could lead to loss of the stability of the system, if not compensated by glycolytic H⁺ production. Thus our theoretical results stress the importance of processes/mechanisms that buffer/compensate for changes in cytosolic proton concentration. In particular, we suggest that the second main role of anaerobic glycolysis, apart from additional ATP supply, may be maintaining the stability of the system at intensive exercise.

Key words: anaerobic glycolysis, computer model, creatine kinase, mitochondria.

INTRODUCTION

Skeletal muscle is the only contractile tissue that can experience significant changes in the cytosolic pH [i.e. the external pH (pH_e) in relation to mitochondria] under physiological, non-pathological conditions. Two main sources of these variations may be quoted. First, at the onset of exercise the creatine kinase (CK)-catalysed reaction is directed towards production of ATP from phosphocreatine (PCr) associated with proton consumption (increase in pH), whereas during recovery from exercise the reverse process takes place; PCr is resynthesized, which is correlated with H⁺ production (a decrease in pH). Second, protons are produced together with lactate in anaerobic glycolysis, which occurs especially at high exercise intensities, leading to a substantial pH decrease.

Changes in pH may be to a large extent buffered/compensated for by several processes. Any pH variation is very effectively buffered by the cytosolic H⁺-buffering pool, comprising mainly proteins, but also other chemical compounds (buffering constant c_{buf} equal to 0.025 M H⁺/pH unit [1]). A deviation of the actual pH_e value from the 'assigned' pH value of about 7.0 is compensated for by efflux/influx of protons from/to the cytosol to/from the blood [1,2] (in fact, only the efflux of protons was measured in these experiments, see below). H⁺ consumption by the CK-catalysed reaction at the onset of exercise can be, at least potentially, compensated for by activation of proton supply from anaerobic glycolysis. The general scheme of the balance of reactions (processes) producing/consuming cytosolic protons is presented in Figure 1. This figure does not involve proton transport through the inner mitochondrial membrane because this transport makes only a small contribution to the proton balance in cytosol.

Several different effects of variations in pH_e on oxidative phosphorylation may be expected. First, a change in pH_e may disturb the pH gradient across the inner mitochondrial membrane [Δ pH; the difference between pH_e and mitochondrial pH (pH_m)], and consequently the protonmotive force (Δ p), which is proportional to Δ pH [3,4]. Δ p has two components, Δ pH and Δ Ψ (the electrical potential), which are uniquely related to each other [3,4]. Δ p is, according to Mitchell's chemiosmotic theory [5], the key 'intermediate' in ATP synthesis, and its value has very strong kinetic influence on the oxidative phosphorylation system as well as on the ATP synthesis and oxygen consumption fluxes [6].

Figure 1 Scheme of processes producing, consuming and buffering cytosolic protons in skeletal muscle

Abbreviations used: AK, adenylate kinase; CK, creatine kinase; Cr, creatine; LT, lactate threshold; PCr, phosphocreatine; pH_e, external pH; pH_m, mitochondrial pH; VO₂, respiration rate; Δ pH, pH gradient across the inner mitochondrial membrane; Δ p, protonmotive force.

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The effect of proton concentration on the equilibrium of the reaction catalysed by CK constitutes another potential kinetic influence of pH_e on oxidative phosphorylation. The PCr/creatine (Cr) ratio 'buffers' the ATP/ADP ratio [7] and any perturbation of CK equilibrium could affect the latter ratio and, as a result, the oxidative phosphorylation flux. CK consumes/produces protons and is influenced by their concentration. For example, intensive consumption of ATP (and PCr) at the onset of exercise causes rapid H^+ consumption, which shifts the equilibrium of the CK-catalysed reaction towards a further decrease in [ATP] and, as a result, further PCr and H^+ consumption. This positive feedback could lead under some extreme conditions to instability of the system (see below).

The pH value influences the value of the phosphorylation potential (the change in the Gibbs free energy of ATP hydrolysis). However, the rate of oxidative phosphorylation depends directly on ADP (and also P_i and ATP) concentration and not on the phosphorylation potential.

Finally, changes in pH could potentially affect the oxidative phosphorylation system via some non-specific physicochemical effects on, for example, protein conformation, fluidity of the inner mitochondrial membrane and so on. Nevertheless, it has been demonstrated that the kinetic properties of oxidative phosphorylation in isolated mitochondria are not influenced by variations in external pH within the physiological range [8–10].

In the present article we study, using a previously developed computer model of oxidative phosphorylation in intact skeletal muscle, the kinetic effect of variations in pH_e on oxidative phosphorylation via the influence on ΔpH (and Δp) across the inner mitochondrial membrane, and on the equilibrium of the CK-catalysed reaction. Our simulations predict that the value of pH has essentially no effect on the steady-state values of fluxes and most metabolite concentrations (except the PCr/Cr ratio). However, sudden acidification/alkalization of cytosol causes a transient decrease/increase in the respiration rate (VO_2) and variations in several metabolite concentrations. Additionally, the computer model shows that the rate (and direction) of pH change accompanying transition from resting steady-state to active steady-state (exercise) may have a profound effect on the kinetic properties (time courses of fluxes and metabolite concentrations) of the transition between these two steady states.

An increase in pH after the onset of exercise slows down the transition from resting steady state to active steady state. In extreme cases, sudden intensive proton consumption by CK after the beginning of high-intensity exercise could lead, if not compensated by glycolytic H^+ production, to instability of the system. Our theoretical findings emphasize the role of buffering/compensation of variations in pH in skeletal muscle cells. Especially, we suggest that the role of anaerobic glycolysis at intensive exercise may be, apart from additional ATP supply, to prevent the system losing stability.

THEORETICAL PROCEDURES

Computer model

A previously developed computer model of oxidative phosphorylation in intact skeletal muscle [11,12] 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 car-

rier, adenylate kinase (AK), CK and ATP usage. The time variations of the metabolite concentrations that 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 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 values of the independent variables. 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 programmes were written in the FORTRAN programming language.

A detailed description of the model is given elsewhere [11]. Below, only the aspects of the model concerning the cytosolic proton balance are discussed. The resting (basic) pH_e is assumed to be 7.0. CK is assumed to work near thermodynamic equilibrium, for reasons already discussed [11]. The apparent equilibrium constant of CK:

$$K_{obs} = K_{ck} \cdot H_e^+ = \frac{ATP_{te} \cdot Cr}{ADP_{te} \cdot PCr} \quad (1)$$

is equal to about 220 under the conditions prevailing in resting muscle ($[Mg^{2+}] = 3\text{--}4\text{ mM}$, $pH_e = 7.0$). In fact, this equilibrium description would be completely sufficient to calculate the PCr/Cr ratio in all conditions on the basis of a current ATP/ADP ratio and pH. However, in order to involve explicitly the proton production/consumption by CK during transition between different steady states, a fully equivalent kinetic description is used where the forward and reverse reaction rates are described by a linear dependence on substrate concentrations:

$$v_{CK} = k_{fCK} \cdot ADP_{te} \cdot PCr \cdot H_e^+ - k_{bCK} \cdot ATP_{te} \cdot Cr \quad (2)$$

where rate constants k_{fCK} and k_{bCK} are high enough to fulfil the closeness-to-equilibrium requirement under all conditions considered.

The values of the rate constants of CK are not fully arbitrary. We checked that a significant decrease (by more than one order of magnitude) of these values leads to an essential slowing down of the time of transition of [PCr] and $[P_i]$ between the resting and active states (see below, e.g. Figures 5C and 5D), which contradicts experimental results. Therefore, the assumption concerning a significant displacement of CK from equilibrium gives incorrect theoretical predictions.

The efflux/influx of protons from/to the cytosol to/from the blood depends linearly on the pH_e [1,2] and is expressed by the following equation:

$$v_{EFF} = k_{EFF}(pH_0 - pH_e) \quad (3)$$

where $pH_0 = 7.0$ and $k_{EFF} = 10\text{ mM} \cdot \text{min}^{-1}$ [1,2].

It must be stressed that only the efflux (and not influx) of protons from muscle cells was measured in experimental investigations. Our assumption concerning the existence of the influx of protons having kinetic properties that are symmetrical to the kinetic properties of proton efflux may seem natural, but it is not obvious at all. We made our assumption partially by analogy with the proton flow through the inner mitochondrial membrane (through respiratory chain complexes, ATP synthase, phosphate carrier and proton leak), where protons can easily flow in both directions depending on the thermodynamic gradient. Nevertheless, the proton flow across the cellular membrane of myocytes seems to be, unlike the proton flow through the inner mitochondrial membrane, a process displaced from thermodynamic equi-

librium (considering the difference in pH and electrical charge on both sides of the myocyte membrane). Therefore, the above analogy is not a strict one. On the other hand, if no proton influx is assumed, the potential alkalization of cytosol after an onset of exercise discussed below would be much greater, and therefore much stronger mechanisms compensating this alkalization would be required. This problem will have to be resolved in the future by appropriate experimental studies. Fortunately, the assumption concerning the existence of proton influx does not affect significantly our general conclusion, and a lack of proton influx would in fact even reinforce our conclusions.

Our original computer model does not take into account glycolytic ATP/H⁺ production. Therefore, here we introduced a very simple kinetic expression for the anaerobic glycolytic proton production above the lactate threshold (LT) [13]. We assume that this production starts above LT and is linearly proportional to the difference between the actual ATP turnover (v_{UT}) and the ATP turnover at LT [$v_{UT(LT)}$]:

$$v_{HGLYC} = k_{HGLYC} \cdot (v_{UT} - v_{UT(LT)}) \quad (4)$$

where $k_{HGLYC} = 0.2$ and $v_{UT(LT)} = 40 \text{ mM} \cdot \text{min}^{-1}$. The value of k_{HGLYC} was adjusted to decrease pH at intensive (but not maximal) exercise by 0.5 pH units, whereas $v_{UT(LT)}$ was estimated from oxygen consumption at LT [13]. This certainly oversimplified description is only aimed at reflecting very roughly the glycolytic proton production at high exercise intensities. It is only used in the simulation presented in Figure 8 (see below).

The buffering of cytosolic proton concentration is described similarly as the buffering of protons in mitochondrial matrix. The efficiency of buffering is expressed as follows:

$$r_{\text{buf}} = c_{\text{buf}}/c_{0e} \quad (5)$$

where c_{buf} , equal to $0.025 \text{ M H}^+/\text{pH unit}$, is the cytosolic buffering capacity and c_{0e} stands for the 'natural' buffering capacity:

$$c_{0e} = (10^{-\text{pH}_e} - 10^{-\text{pH}_e - d\text{pH}})/d\text{pH} \quad (6)$$

where $d\text{pH}$ is a small change in pH_e . c_{0e} is equal to approx. $2.3 \times [\text{H}^+]$.

The overall cytosolic proton production/consumption is described as follows:

$$\dot{H}_e^+ = (v_{\text{OXPHOS}} - v_{\text{CK}} - v_{\text{EFF}} + v_{\text{HGLYC}})/r_{\text{buf}} \quad (7)$$

where v_{OXPHOS} stands for transport of protons across the inner mitochondrial membrane and is associated with the following processes (see [11] for details): pumping of protons outside mitochondria by complexes I, III and IV of the respiratory chain, ATP synthesis coupled with return of protons to mitochondria, transport of ATP/ADP and P_i , driven by proton gradient, as well as proton leak. v_{OXPHOS} has only an insignificant effect on the proton balance in cytosol and therefore it is not taken into account in Figure 1. In some simulations pH_e was kept constant and therefore no changes in proton concentration took place:

$$\dot{H}_e^+ = 0$$

Simulations

The energy demand (the rate constant of ATP usage, k_{UT}) was fixed at a constant value in the simulations presented in Figures 2–4 (see below). The relative energy demand (standardized at 1 in the resting state) was equal to 5 (small energy demand) in the simulations presented in Figures 2 and 4, or 15 (medium energy demand) in the simulations presented in Figure 3. In the simulations presented in Figure 5 (see below), the rate constant

of ATP usage was increased after 3 min of simulation from the resting value (relative energy demand, 1) to a value 15 times greater than the resting value (medium energy demand). In the simulations presented in Figures 6–8 (see below) the relative energy demand was increased after 3 min of simulation from 1 (resting value) to 100 (high energy demand). Since, as discussed previously [11,12,14], at high work intensities not only ATP usage but also the ATP-producing block is most probably activated directly to allow for a very high increase in VO_2 and ATP turnover, accompanied by only moderate changes in the ATP/ADP ratio, in the simulations shown in Figures 6–8 (see below) all the oxidative-phosphorylation steps were activated (their rate constants increased) seven times, in parallel with the 100-fold activation of ATP usage.

One should bear in mind that the relative increase in the VO_2 in the active state in relation to the resting state is less than half the relative increases in energy demand and ATP turnover. For example, 5-, 15- and 100-fold increases in energy demand lead to 2.4-, 6.2- and 43-fold increases in VO_2 , respectively (see Figures 2B, 3B, 4B, 5B and 6A, below). This is because proton leak accounts for over 50% of oxygen consumption in the resting state, the remaining proportion being due to ATP synthesis [15].

In the simulations presented in Figures 2–4, aimed at analysing the effect of a sudden externally imposed large acidification/alkalization of cytosol on oxidative phosphorylation, pH_e was kept constant for the first 3 min of simulation, and then instantly changed to a new constant value (acidification by 1.0 pH unit, Figures 2 and 3, or alkalization by 0.5 pH units, Figure 4). In the simulations presented in Figure 5, pH_e was either kept constant or allowed to undergo changes resulting from proton consumption by CK and influx of protons from blood to cytosol. The proton production in anaerobic glycolysis was turned off in these simulations. In the simulation shown in Figure 6, pH_e was kept constant at 7.0. In the simulation shown in Figure 7, pH_e changed after the onset of exercise as a result of proton consumption by CK and proton influx from the blood. In the simulation shown in Figure 8, pH_e changed after the onset of exercise as a result of proton consumption by CK, proton influx and glycolytic proton production.

THEORETICAL RESULTS AND DISCUSSION

Effect of sudden externally caused acidification or alkalization

First, we analysed the effect of a sudden (instant) externally imposed acidification or alkalization of cytosol on the kinetic properties of oxidative phosphorylation remaining in one particular set of conditions (with a given constant energy demand). Such an event most probably does not reflect exactly any realistic physiological situation. However, we aimed to study the 'pure' effect of pH change, not 'contaminated' with any additional changes, in particular variations in energy demand. Additionally, the predictions given by our simulations can be tested in artificial experimental conditions.

Figure 2 presents the simulated effect of a sudden drop in pH_e by 1 pH unit (from 7.0 to 6.0) at low relative energy demand (5 times greater than in the resting state). One can see that the initial steady state is disturbed for about 10 min (transition state) and that after this time a new steady state is achieved. Figure 2(A) shows that ΔpH (and consequently Δp) increases temporarily in the transition state, but returns ultimately to the initial value. This means that the pH_m passively adjusts its value to the pH_e and that the proton gradient remains unchanged in the new steady state in relation to the initial steady state. As a result, the value of VO_2 is not affected by changes in Δp in the new steady state.

This is demonstrated in Figure 2(B), where one can see that VO_2 returns to its initial value after the transient state. Nevertheless, a large transient decrease in oxygen consumption takes place after sudden acidification of cytosol. This is caused by the fact that a decrease in pH causes a shift in the CK-catalysed reaction towards an increase in the ATP/ADP ratio. As a result, the concentration of ADP, the main metabolite activating oxidative phosphorylation, decreases, which inhibits the VO_2 . High [ADP] is slowly restored as the result of conversion of PCr to Cr, which leads to a decrease in the PCr/Cr ratio. During this conversion ATP consumed by ATP usage is resynthesized mainly from PCr and therefore ATP supply from oxidative phosphorylation may be low. However, when a new steady state is achieved, with a new equilibrium of the CK-catalysed reaction, fluxes and metabolite concentrations also stabilize at new constant values. VO_2 returns to its initial value. The final ATP/ADP ratio is a little different from the initial one because of some increase in the P_i concentration (produced as a result of PCr decomposition; results not shown). On the other hand, the new PCr/Cr ratio is significantly lower than the old one. In fact, this is the only parameter that has changed to a considerable extent after transition to a new steady state with a decreased pH. Nevertheless, this fact does not influence the VO_2 , since this rate depends directly on [ADP], [ATP] and P_i , and not on [Cr] and [PCr] (see below).

The transient increase in ΔpH shown in Figure 2(A) is a direct result of the transient change in the ATP/ADP ratio (and P_i),

Figure 2 Simulated effect of a sudden externally caused decrease in pH_e by 1 pH unit at low exercise intensity

Energy demand five times greater than in the resting state. pH was suddenly decreased from 7.0 to 6.0 after 3 min of simulation. (A) Variations over time of pH_e, pH_m and ΔpH . (B) Variations over time of VO_2 , ATP/ADP and PCr/Cr.

Figure 3 Simulated effect of a sudden externally caused decrease in pH_e by 1 pH unit at medium exercise intensity

Energy demand 15 times greater than in the resting state. pH was suddenly decreased from 7.0 to 6.0 after 3 min of simulation. Details are as for Figure 2.

since Δp is strictly related to the mitochondrial phosphorylation potential via the near-equilibrium reaction catalysed by ATP synthase. In the absence of the CK/PCr/Cr system, changes in pH_m very quickly (in fact, almost instantly) follow passively any variations in pH_e, and ΔpH (as well as Δp) remains essentially constant (simulations not shown).

The transition time in the above simulation for low relative energy demand (equal to 5) was rather long (about 10 min) for two reasons. First, at low ATP turnover it takes time to convert a part of PCr into Cr as the result of resynthesis of ATP consumed by ATP usage, and thus to reach a new steady-state PCr/Cr ratio. Second, the PCr concentration itself is high at low energy demands and therefore more PCr must be converted to Cr in order to achieve a new steady state. These two effects are additive and therefore one could expect a greater than proportional decrease in the transition time with an increase in energy demand (ATP turnover). This is demonstrated in Figure 3, where a simulation for a three-times-greater relative energy demand than in the previous simulation, namely 15, is presented. The qualitative behaviour of the system after a sudden drop in pH_e is similar to that in Figure 2. However, the transition time is much shorter here, equal to about 2 min. Therefore, as expected above, a 3-fold increase in energy demand (ATP turnover) causes a non-proportionally large 5-fold decrease in the transition time.

The transition time in the simulation presented in Figure 2 is long also because acidification causes a shift in the CK-catalysed reaction in the direction of a higher ATP/ADP ratio in relation to the PCr/Cr ratio, which is associated with a transient decrease in [ADP] and, consequently, with inhibiting oxidative phosphorylation. One could expect that sudden alkalization should have

Figure 4 Simulated effect of a sudden externally caused increase in pH_e by 0.5 pH units at low exercise intensity

Energy demand five times greater than in the resting state. pH was suddenly increased from 7.0 to 7.5 after 3 min of simulation. Details are as for Figure 2.

the opposite effect; it should cause a transient increase in [ADP], VO_2 and oxidative ATP production. This should speed up reaching a new steady state. The simulation presented in Figure 4 demonstrates that this actually takes place. This simulation predicts the effect of sudden alkalization of the cytosol by 0.5 pH units (alkalization by 1 pH unit would be completely irrelevant for physiological conditions) for a relative energy demand equal to 5, as in the simulation presented in Figure 2. One can see that changes in fluxes and metabolite concentrations proceed here, as expected, in the opposite direction to those in Figure 2; VO_2 transiently increases and then returns to its initial value, ΔpH transiently decreases, the PCr/Cr ratio increases and ATP/ADP ratio decreases (the changes in ATP/ADP are greater here because of redistribution between mitochondrial and cytosolic P_i and a decrease in the overall P_i pool, which is compensated for by a decrease in ATP/ADP; results not shown). However, the transition time is much shorter here, equal to about 1 min, compared with 10 min in Figure 2. Of course, this is partially due to the fact that pH was increased here by 0.5 pH unit and not by 1 pH unit. Nevertheless, it can be seen clearly that the effect of alkalization on oxidative phosphorylation is not a mirror image of the effect of acidification.

The simulations presented in Figures 2–4 demonstrate some general rules. First, the transition time between the steady state before and the steady state after pH change depends inversely on energy demand (ATP turnover): the greater the ATP turnover, the shorter the transition time. Second, acidification transiently blocks oxidative ATP production and therefore lengthens the transition time, whereas alkalization transiently activates oxidative phosphorylation, thus shortening the transition time.

Third, VO_2 changes only transiently after sudden pH change and then returns to its initial value. The same property concerns ΔpH (and Δp) across the inner mitochondrial membrane and, to a somewhat lesser extent (at least in the case of alkalization), the ATP/ADP ratio. On the other hand, the PCr/Cr ratio significantly decreases after acidification and increases after alkalization.

A significant displacement of CK from equilibrium (a decrease in the value of its rate constants) would lengthen the transition time between the steady states before and after pH change. However, such a displacement is not likely, as discussed here and in [11].

It must be emphasized that the above predictions are valid under the assumption that ADP (and P_i) is the main metabolite that directly activates oxidative phosphorylation in mitochondria. Nevertheless, some authors propose that Cr is the main direct activator of oxidative phosphorylation [16]. In such a case, the expected effect of pH changes would be the opposite to that presented above; acidification would transiently increase VO_2 and cause a persistent increase in the ATP/ADP ratio (the PCr/Cr ratio would transiently decrease, but afterwards it would return to its initial value), whereas alkalization would transiently decrease VO_2 and cause a persistent decrease in the ATP/ADP ratio (PCr/Cr ratio would increase transiently, but then return to its initial value; simulations not shown). Also, transition time would be shorter in the case of acidification than in the case of alkalization. Therefore, the above simulations seem to offer an attractive experimental test for deciding which metabolite is the main direct activator of oxidative phosphorylation: ADP or Cr (one must bear in mind that such an experiment would have to be conducted in a system containing both oxidative phosphorylation with CK/PCr/Cr and ATP usage relatively independent of the ATP/ADP ratio).

The above proposal is in a sense a dynamic version of the steady-state argument proposed by Connet [17]. The advantage of our method is that it involves an easy-to-measure variable, namely oxygen consumption, which returns to its initial value when a new steady state is reached (and therefore cannot be used in a steady-state test). The test proposed by us probably could be performed in skinned fibres in the presence of PCr/Cr and an artificial ATP-usage system.

Effect of proton consumption by CK after the onset of exercise

The sudden, externally imposed changes in pH_e do not normally take place in intact muscle under physiological conditions. In skeletal muscle *in vivo* changes in pH are associated with variations in energy demand. At low and medium work intensities, below the LT, the main process that may disturb the resting pH value is thought to be the CK-catalysed reaction which consumes protons after the onset of exercise and produces protons during recovery from exercise. Variations in proton concentration are buffered by the cytosolic H^+ -buffering pool and are compensated for by influx/efflux of protons, and possibly also by some other processes. The role of glycolytic proton production is not clear.

In the simulations presented in Figure 5 we studied the effect of alkalization of the cytosol as a result of proton consumption by CK after the onset of exercise on the time courses of VO_2 and different metabolite concentrations during the transition from the resting to the active steady state. To do this, we compared the results of two simulations. In the first simulation (marked by solid lines in Figure 5), it was assumed that pH_e did not change at all because of a very efficient proton buffering, very quick influx/efflux of protons and/or a perfect compensation of proton

Figure 5 Simulated transition times at constant and varying pH

Comparison of time courses of four variables, pH_e (A), VO_2 (B), $[\text{PCr}]$ (C) and $[\text{P}_i]$ (D), after the onset of medium-intensity exercise (relative energy demand, 15) between the case with constant pH (solid line) and the case where pH changes as a result of the balance between proton consumption by CK and proton influx from blood (dashed line). No glycolytic proton production is assumed.

consumption by CK by proton supply from anaerobic glycolysis. In the second simulation (marked by dashed lines in Figure 5), we used the values of H^+ -buffering and influx/efflux activity taken from the literature [1,2], and assumed that no anaerobic glycolysis takes place below LT.

In Figure 5(A) the time courses of pH_e obtained in both simulations are compared. Of course, pH_e was constant in the first simulation, in which a perfect buffering/compensation of variations in proton concentration was assumed. In the second simulation, in which pH changed due to the balance between proton consumption by CK and proton influx from blood, pH_e first increased by over 0.2 pH units after the onset of medium-intensity exercise (H^+ consumption by CK), and then started to return to the initial value (influx of H^+ from blood). Both the extent and the duration of the alkalization predicted by this simulation were significantly greater than the extent and duration of initial alkalization observed in experiments after the onset of exercise [1,18–20] (in [18], for example, pH rises by only 0.1 pH units for only 0.5 min). It is not clear if such a great discrepancy between experiments and simulations, which were based on values of proton buffering and efflux taken from the literature [1,2], can be explained by variation in these values between different muscles and experimental procedures [21], or if some additional factor is needed. This factor could be activation of glycolytic H^+ production by alkalization of the cytosol, even below the LT.

Such a possibility has already been proposed by several authors. Brooks [22] suggested that below the LT activation of anaerobic glycolysis is too low to cause noticeable accumulation of lactate in blood. Gollnick et al. [23] as well as Ivy et al. [24] postulated recruitment of some of the glycolytic type II muscle fibres, even at low exercise intensities well below the LT.

Figure 5(B) compares the time courses of VO_2 in both simulations. It can be seen that the initial alkalization after the onset of exercise first speeds up the increase in the VO_2 , but afterwards VO_2 grows slower than when pH is constant. Therefore, generally, the initial alkalization slows down the transition of oxygen consumption to a new, active steady-state value. This effect is even clearer and more pronounced for time courses of the decrease in $[\text{PCr}]$ and increase in $[\text{P}_i]$ during the transition state (Figures 2C and 2D). Again, the simulation with constant pH fits better to experimental data [25,26] than the simulations giving significant initial alkalization after the onset of exercise. And again, this may be explained potentially by the fact that proton production by anaerobic glycolysis (and its activation by, for example, elevated $[\text{AMP}]$ or pH) even below the LT is not taken into account by the model (although other explanations, e.g. variability in different muscle types and/or experimental results, cannot be excluded). This hypothesis is confirmed by the observation that pH increases significantly after an onset of exercise in people with McArdle's disease, who have defective glycogenolysis and glycolysis [1].

Figure 6 Simulated variations over time of chosen variables after the onset of high-intensity exercise after 3 min of simulation with constant pH_e .

Relative energy demand was equal to 100.

Interestingly, the transition time for $[ADP]$ ($[ATP]$ is essentially constant in the above simulations) is shorter in the simulation with varying pH than in the simulation with constant pH (results not shown). Therefore, $[ADP]$ seems to be an exception among the studied parameters.

Generally, the above simulations demonstrate the significance of buffering/compensation of variations in pH for speeding up the transition from the resting to the active steady state, which seems to be profitable from the point of view of a possibly fast response to an increase in energy demand (especially in the case of a rapid increase in respiration and ATP synthesis). On the other hand, the lengthened transition time for ADP could be harmful for muscle contraction (actinomyosin-ATPase) provided that this process is sensitive to $[ADP]$. However, ATP usage in muscle does not seem to be very sensitive to the ATP/ADP ratio and therefore the significance of the transition time for ADP is not clear.

Different intensities of anaerobic glycolysis, proton buffering and/or proton influx/efflux in different muscle types can cause different time courses of VO_2 and metabolite concentrations in transition state.

It must be stressed that in the absence of proton influx the lengthening of the transition time caused by the alkalization of cytosol by CK would be even greater than in the above simulations, because the alkalization itself would be greater (theoretical results not shown). Any significant displacement of the CK-catalysed reaction from thermodynamic equilibrium would significantly lengthen the transition times for PCr and P_i (results not shown), which would contradict the experimental studies. This fact further supports our assumption that CK works near equilibrium.

Figure 7 Simulated variations over time of variables after the onset of high-intensity exercise after 3 min in which pH varies as a result of the balance between proton consumption by CK and proton influx from blood.

Relative energy demand was equal to 100. No glycolytic proton supply was assumed.

Effect of glycolytic proton supply at high exercise intensity

The above simulations suggest that production of protons in anaerobic glycolysis could potentially play some role in compensating the alkalization of the cytosol caused by H^+ consumption by CK, even at low- and medium-intensity exercise below the LT. A much more significant effect of glycolytic proton supply on oxidative phosphorylation can be expected above the LT, especially with intensive exercise, where the oxidative capacity for ATP production is nearly exhausted and a significant fraction of ATP is supplied by anaerobic glycolysis, which is accompanied by a significant acidification of cytosol.

In order to analyse the effect of different processes consuming/producing protons under intensive exercise we made three simulations for a large increase in relative energy demand from 1 (the resting state) to 100 (intensive exercise). In the first simulation, presented in Figure 6, pH_e was kept constant. One can see that after the onset of exercise a new active steady state is quickly achieved, with new VO_2 and metabolite concentrations (Figure 6A). ΔpH stabilizes at a new lower level, corresponding to the new value of mitochondrial phosphorylation potential (Figure 6B).

In the second simulation, presented in Figure 7, only proton consumption by CK and efflux/influx of protons, but not glycolytic proton supply, were taken into account. The behaviour of the system seems to be very strange in this case, as can be seen in Figure 7(A): the system completely loses its stability for about 5 min. It was carefully checked that this unexpected theoretical result was not due to inaccuracy of the numerical procedures.

Scheme 1 Explanation for the instability in the system

Upper panel: the system in which instability takes place. Lower panel: the sequence of events leading to the instability. Details are given in the text. O.P., oxidative phosphorylation; UT, ATP usage; ↑, increase; ↓, decrease.

used, but constituted an intrinsic property of the system. The unstable behaviour observed was caused by a sort of a positive feedback present in the system. Namely, a sudden conversion of PCr to Cr (as a result of rapid ATP hydrolysis) occurring at the onset of intensive exercise caused a quick consumption of protons and an increase in pH. This in turn further shifted the equilibrium of the CK-catalysed reaction towards a further decrease in [ATP] and thus further PCr and proton consumption. As a result, the initial signal underwent amplification by the mechanism of positive feedback. Figure 7(B) shows that this unstable behaviour was associated with (or, in fact, initiated by) a steep increase in pH_e after the onset of intensive exercise. Of course, such instability of behaviour would be very harmful for an organism, because it would be unable to perform any intensive exercise and might lead to cell death because of a great transient decrease in the ATP/ADP ratio.

The kinetic mechanism underlying the instability is presented in more detail in Scheme 1. The components of the system that are important for the occurrence and kinetic properties of the instability are: oxidative phosphorylation, ATP usage, CK and AK (see Scheme 1, upper panel). The sequence of events leading to the instability may be explained as follows (Scheme 1, lower panel). A great increase in ATP usage taking place at the onset of intensive exercise initiates a sudden decrease in the ATP/ADP ratio and, via the very quick reaction catalysed by CK, leads to a rapid decrease in the PCr/Cr ratio. Conversion of PCr to Cr involves rapid consumption of protons and alkalization of cytosol. An increase in pH stimulates a further decrease in the ATP/ADP ratio, which leads to positive feedback (stimulation of a reason by its result). AK catalyses the reversible conversion of two ADP molecules into one ATP molecule and one AMP molecule; therefore, a decrease in ATP/ADP causes conversion of some ADP to AMP and ATP. The latter compound is quickly consumed by high energy demand, which leads to further AMP production. However, the instability would not appear without sudden variations in pH, responsible for the self-stimulation of

Figure 8 Simulated variations over time of variables after the onset of high-intensity exercise after 3 min in which pH varies as a balance between proton production by anaerobic glycolysis, proton consumption by CK and proton efflux/influx to/from blood

Relative energy demand equal to 100.

the decrease in ATP/ADP (results not shown). In a sense, the instability is caused by a kind of a large 'overshoot' in the CK system. This overshoot could be diminished by a decrease in the rate constants of CK, but this would significantly slow down the transition between different steady states (see above).

It is worth stressing that in the absence of the proton influx the instability of the system would be even more pronounced and would appear at lower work intensities (theoretical results not shown). Nevertheless, one must bear in mind that instabilities are generally very sensitive to parameter values and system properties, and that kinetic models of metabolic systems are only approximations of complex reality. Therefore, the above-discussed theoretical possibility of the appearance of instability will have to be tested in experimental studies.

This serious problem of the possible instability can be overcome if proton production by anaerobic glycolysis, occurring at intensive exercise above LT, is taken into account. In the simulation presented in Figure 8 both H^+ consumption by CK and H^+ production by glycolysis are involved. One can see that glycolytic proton supply compensates partially the rapid initial alkalization of cytoplasm caused by CK, and thus prevents the instability from appearing. There is only a small overshoot in [ADP] and pH_e . On the other hand, the continuous proton supply from anaerobic glycolysis causes continuous changes in [PCr], [P_i] and VO_2 and prevents the reaching of a steady state after the onset of high-intensity exercise (the metabolite concentrations continue to change in the same direction until exhaustion of muscle and termination of exercise).

It must be emphasized that a very simplified kinetic description of glycolytic proton production was used in this simulation. For example, this description does not take into account any activation/inhibition of glycolysis by low/high proton concentrations (most probably the overshoot in [ADP] would vanish if a more realistic kinetic description was used in the above simulation). Nevertheless, this description reflects roughly (qualitatively) the main kinetic effects of cytosolic acidification caused by continuous supply of protons from anaerobic glycolysis. First, pH decreases by 0.5 units during 12 min of intensive exercise, which seems to be a realistic estimation for submaximal exercise where oxidative phosphorylation is still the main source of ATP (the decrease in pH slows down with time, since at lower pH values the rate of proton efflux increases). Second, the instability of the system is avoided. This suggests that, at least in principle, the second main role of anaerobic glycolysis, apart from additional ATP supply, may be to maintain the stability of the system. Third, the effect of continuous glycolytic proton supply on oxidative phosphorylation can account for some part of the phenomenon of the slow component in oxygen-uptake kinetics [27], as proposed by some authors [27,28], since VO_2 slightly increases with time in Figure 8(A).

The model of energy metabolism used in the present article is rather complicated; in particular, it has an extensively developed oxidative phosphorylation part. Some (but certainly not all) of the above-presented theoretical results probably could be obtained with the aid of simpler models, although presumably after minor modification; one example is the model developed by Connet et al. [17,29]. This model involves the equilibrium expressions for CK and AK, and should be able, when used appropriately, to demonstrate even the above-described instability, although it would not be able to simulate, for example, the changes in pH_m and Δp . The non-linearity causing this instability is not explicitly incorporated into the present model or Connet's model, but it appears under some extreme conditions as a result of the kinetic properties of the rapid reaction catalysed by CK.

Some other models (e.g. that developed by Meyer [25]) are too simple to be used for studying the problems dealt with in the present study, lacking for example proton production/consumption and AK.

Conclusions

To sum up, in the absence of the CK/PCr/Cr system variations in pH_e exert essentially no effect on oxidative phosphorylation via changes in ΔpH (or Δp). This is because pH_m very quickly and passively follows changes in pH_e , so that ΔpH remains unchanged. In the presence of the CK/PCr/Cr system, all transient changes in ΔpH (and Δp) are not caused by variations in pH_e directly, but indirectly via changes in ATP/ADP and $[\text{P}_i]$.

The shift in the equilibrium of the CK-catalysed reaction caused by variations in pH_e has, by itself, essentially no effect on the steady-state values of VO_2 and ΔpH , a small effect on the steady-state value of ATP/ADP and a significant effect on the steady-state value of PCr/Cr. Sudden externally imposed acidification/alkalization of cytosol causes a large transient decrease/increase in the VO_2 , an increase/decrease in ATP/ADP and an increase/decrease of ΔpH . The transition time depends on ATP turnover and on the extent and direction of the change in proton concentration.

Alkalization of cytoplasm by the CK-catalysed reaction after the onset of low- and medium-intensity exercise (below the LT) slows down the transition from the resting to the active state. During high-intensity exercise (well above the LT) the con-

sumption of protons by CK could lead, in the absence of glycolytic proton production, to instability of the system. Therefore, it is very important for a muscle cell to buffer/compensate for the initial alkalization after the onset of exercise and one of the main roles of anaerobic glycolysis may be to perform this task.

pH_e may, directly or indirectly, affect the oxidative phosphorylation system via other ways than those studied in the present article. For example, acidification of muscle during intensive exercise could decrease the efficiency (increase the energetic costs) of mechanical work or exert some unspecific physicochemical effect on mitochondria. However, the latter possibility is not likely, since external pH has no effect on oxidative phosphorylation in isolated mitochondria within the physiological pH range [8–10].

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