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Theoretical studies on the regulation of anaerobic glycolysis and its influence on oxidative phosphorylation in skeletal muscle

Bernard Korzeniewski*, Piotr Liguzinski

Institute of Molecular Biology and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387, Kraków, Poland

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Abstract

It is shown, using the computer model of glycolysis in skeletal muscle developed recently by Lambeth and Kushmerick (Ann. Biomed. Bioenerg, 30 (2001) 19-34) incorporated into the computer model of oxidative phosphorylation developed by Korzeniewski et al. (Biophys. Chem. 83 (2001) 19-34) that the regulation of glycolysis by ADP, AMP and P_i is decidedly insufficient to explain the large increase in the glycolytic flux during transition from rest to intensive exercise in intact skeletal muscle. Computer simulations based on a simple kinetic description of the glycolytic ATP and H^+ production strongly suggests that glycolysis must be directly activated during muscle contraction. They also demonstrate that the inhibition of glycolysis by H^+ is needed to explain the transient activation of this pathway at the onset of exercise as well as the duration time and extent of the initial alkalization after the onset of exercise. Finally, it is shown that ATP supply from anaerobic glycolysis slows down the V_{O_2} kinetics during rest-to-work transition.

Keywords: Glycolysis; Glycogenolysis; Skeletal muscle; Computer model; Cytosolic pH; Regulation of metabolism

1. Introduction

Muscle contraction is driven by the hydrolysis of ATP to ADP and inorganic phosphate. ATP is mostly consumed by actinomyosin-ATPase (approx. 70% of ATP usage in working muscle) and calcium-ATPase (approx. 30% of ATP usage in working muscle). There are three sources of ATP in the skeletal muscle cell: oxidative phosphorylation, creatine kinase and anaerobic glycolysis. Oxidative phosphorylation (plus aerobic glycolysis and TCA cycle) is the main ATP supplier in the resting state and during steady-state exercise in a broad range of intensity (from light to intensive exercise) in mitochondria-rich (type I and IIA) muscle fibers. The conversion of PCr to Cr catalyzed by creatine kinase (CK) constitutes the predominating ATP-producing process in the initial phase of exercise, during first seconds or tens of seconds (depending on the intensity of exercise) of muscle activity [1,2]. Finally, a transient recruitment of ATP synthesis by anaerobic glycolysis takes place during first seconds/minutes of exercise [1–4]. Anaerobic glycolysis is also the main source of ATP during extremely intensive, short-term muscle work [1–3].

* Corresponding author. Tel.: +48-12-26-63-73; fax: +48-12-26-69-02.
E-mail address: benio@mol.uj.edu.pl (B. Korzeniewski).

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An important factor influencing the kinetic properties of the bioenergetic system in skeletal muscle is the cytosolic proton concentration [5]. H^+ ions are produced/consumed by several processes. They are consumed by creatine kinase (CK) during the initial stage of exercise, what may lead to a transient alkalization of muscle, and this enzyme produces them during muscle recovery. Protons are produced together with lactate/pyruvate by glycolysis and consumed when lactate/pyruvate is consumed by oxidative phosphorylation. In particular, the net production of protons takes place during anaerobic glycolysis, where the glycolytic lactate/pyruvate/H^+ production predominates over the consumption of lactate/pyruvate/H^+ by oxidative phosphorylation. Protons can be also transported to/from blood through the cell membrane by the efflux/influx of protons (to be sure, only the efflux of protons has been measured experimentally) [6]. Finally, the concentration of hydrogen ions in the cytosol is very effectively buffered by the cytosolic H^+-buffering pool (mostly different proteins) [6]. The network of particular processes that produce, consume and buffer cytosolic ATP and protons is presented schematically in Fig. 1.

The kinetic properties of particular processes responsible for ATP delivery, as well as the kinetic interplay between these processes, are essential for our understanding of the quantitative behavior of the bioenergetic system in skeletal muscle, and of its regulation in response to an increased energy demand. Among others, it is important to know

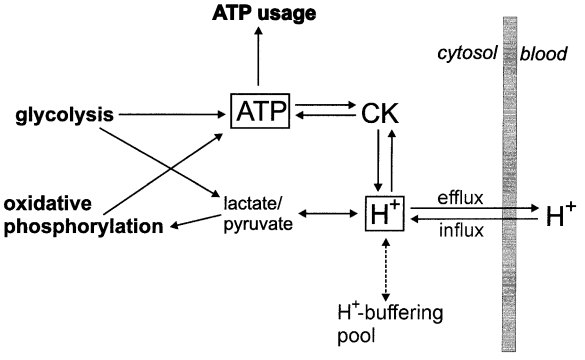

Fig. 1. Scheme of processes producing, consuming and buffering cytosolic ATP and H^+ in skeletal muscle.

what are the changes in cytosolic pH under different physiological conditions, and what effect the proton concentration exerts on different elements of the muscle bioenergetic system. In particular, the regulatory mechanisms responsible for adjusting the intensity of the glycolytic flux under different conditions and energetic states to the current physiological need still remain a matter of debate [3]. The rate of glycolysis may increase 100 times or more during transition from resting state to intensive exercise [3,7]. Anaerobic glycolysis leads to an acidification of the cytosol by 0.1–0.2 pH units at moderate exercise and by approximately 0.5–0.7 pH units at intensive exercise [3,6,8–10]. It is well established that glycolysis is activated by metabolites the concentration of which increases during muscle contraction: ADP, AMP and P_i, and is inhibited by low pH [3]. It has been also postulated that some still unknown factor, related to muscle contraction, directly activates glycolysis [3,7]. However, the quantitative contribution of these factors to the general behavior of the system remains unclear. Therefore, the kinetic mechanisms underlying the dynamic properties of glycolysis in skeletal muscle are still rather poorly understood.

The computer model of oxidative phosphorylation in isolated skeletal muscle mitochondria developed previously and broadly tested by comparison with various experimental data [11,12] was next extended in order to include some components of the bioenergetic system of an intact skeletal muscle cell, in particular the CK/PCr/Cr system, efflux/influx of protons and buffering of protons in cytosol [13]. This model was also able to match correctly experimental data and was subsequently used for theoretical investigations concerning the effect of rapid changes in cytosolic pH on the oxidative phosphorylation system in muscle [5].

Nevertheless, this model still lacked one very important element necessary to complete the quantitative description of the bioenergetic system in skeletal muscle, namely the third main ATP supplier—anaerobic glycolysis. This was partly due to the fact that no reliable, comprehensive model of glycolysis in skeletal muscle was available in the literature (computer models of this process in red blood cells were inappropriate for this purpose,

since it is very likely that the regulation of glycolysis in muscle is very different than in red cells). Fortunately, a comprehensive and reliable model of glycolysis in skeletal muscle was published recently by Lambeth and Kushmerick [14]. Within this model, the dependence of the rates of the reactions catalyzed by particular glycolytic enzymes on the concentrations of intermediate metabolites as well as of some external regulators such as ADP, AMP, ATP and P_i is described by a set of appropriate kinetic equations that were taken from the literature. The model allowed to produce several interesting theoretical results; for instance, it predicted that the glycolytic flux in muscle (in the absence of oxidative phosphorylation) is controlled mostly by ATP usage, while within the small control kept by glycolysis the main controlling steps were glycogen phosphorylase (GP) and (to a less extent) phosphofructokinase (PFK). However, the discussed model does not involve the direct activation of particular glycolytic enzymes during muscle contraction and the dependence of the glycolytic flux on H^+ concentration. Several glycolytic enzymes are significantly inhibited by low pH ([3,15], see also below). At the same time, significant changes in pH may take place in skeletal muscle during transitions between different energetic states.

The first goal of the present work was to incorporate the model of glycolysis developed by Lambeth and Kushmerick [14] to the model of oxidative phosphorylation in skeletal muscle developed previously by Korzeniewski et al. [5,11] and to check if such a joined model is able to account for the kinetic properties of glycolysis in skeletal muscle, especially for the large increase in the glycolytic flux during transition from work to intensive exercise. Because this appeared to be decidedly not the case, the next aim was to develop a simple kinetic description of the dependence of the glycolytic flux in muscle on ADP, AMP, ATP and P_i, based on the model of glycolysis developed by Lambeth and Kushmerick, to supplement this description with the direct activation of glycolysis and/or with the inhibition of glycolysis by protons, and to incorporate this description to the previously developed model of oxidative phosphorylation. Next, it was intended to check if the direct activation of glycolysis plus regulation of this process by adenine nucleotides and inorganic phosphate can account satisfactorily for the kinetic properties of glycolysis in intact muscle, or if the inhibition by protons is also needed. The last aim was to investigate the effect of the presence of anaerobic glycolysis on the kinetic properties of oxidative phosphorylation and of the whole bioenergetic system in skeletal muscle.

The present theoretical study strongly suggests that the direct activation of glycolysis is responsible for the large increase in the glycolytic flux during rest-to-work transition and that the inhibition/activation of the glycolytic flux by acidification/alkalization is necessary to explain the transient activation of glycolysis after an onset of exercise and the extent and duration of the initial alkalization encountered in experimental studies. Computer simulations also show that anaerobic glycolysis delays the transition between resting V_{O_2} and active V_{O_2} (although it does not affect significantly the transition time of [PCr] and [P_i]), decreases the initial alkalization at the beginning of exercise and is able to prevent the instability in the system, which could otherwise appear at intensive work intensities [5]. Generally, it is proposed that the regulation of glycolysis in the range of the energetic states studied in the present work (from rest to intensive exercise, in which oxidative phosphorylation is still the main ATP-supplying process) can be explained as a result of the 'competition' or balance between the activation of this pathway by some cytosolic factor related to muscle contraction, by ADP+AMP+P_i and by alkalization, and the inhibition of glycolysis by acidification.

2. Theoretical procedures

2.1. Computer model

In the first part of the present work, the comprehensive model of glycolysis in skeletal muscle developed by Lambeth and Kushmerick [14] was incorporated into the model of oxidative phosphorylation in intact skeletal muscle developed by Korzeniewski et al. [5,11].

The computer model of oxidative phosphorylation in intact skeletal muscle developed previously [5,11] contains explicitly the following enzymes/processes/metabolic blocks: substrate dehydrogenation (hydrogen supply to the respiratory chain), complex I, complex III, complex IV (cytochrome *c* oxidase), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, adenylate kinase, creatine kinase, ATP usage, proton efflux/influx from/to muscle. 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) were expressed in the form of a set of ordinary differential equations. The other (dependent) variable values (other metabolite concentrations, thermodynamic forces, etc...) were calculated from the independent variable values. The set of differential equations was integrated numerically. In each iteration step, new values of rates, concentrations and thermodynamic forces were 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.

Recently, a comprehensive model of glycogenolysis and glycolysis in skeletal muscle was developed by Lambeth and Kushmerick [14]. Within this model, the dependence of the rates of particular glycolytic enzymes on different metabolites (internal metabolites of glycolysis and external metabolites such as ADP, AMP, ATP and P_i) was described by appropriate kinetic equations. This model proved to be a very useful tool for theoretical quantitative studies of the control and regulation of glycolysis [14]. However, the discussed model involves neither the postulated direct activation of glycolysis during muscle contraction nor the inhibition of this process by protons.

In order to incorporate the model of glycolysis into the model of oxidative phosphorylation, the former model was first implemented and started separately to check if it is able to reproduce the theoretical results presented in the original paper [14]. Next, some minor modifications were introduced to this model in order to make it compatible with the model of oxidative phosphorylation. First, it was assumed that the cytosolic $NADH/NAD^+$ ratio is constant; this approximate assumption is justified by the relative stability of the $NADH/NAD^+$ ratio encountered in intact skeletal muscle and by the fact that oxidative phosphorylation consumes NADH, what was not the case in the original model of 'isolated' glycolysis [14]. Second, it was assumed, for obvious reasons, that pyruvate is consumed by oxidative phosphorylation, and that in resting state the rate of pyruvate consumption is equal to the rate of pyruvate production (no anaerobic glycolysis). Third, the arbitrary value of the rate constant of the lactate efflux from muscle cell to blood fixed by Lambeth and Kushmerick in order to prevent the accumulation of lactate in the cytosol was decreased 10 times in order to minimize anaerobic glycolysis in resting state. Fourth, the Michaelis–Menten constants of GP (glycogen phosphorylase) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were increased 10 times in order to avoid a decrease in P_i concentration below 0.5 mM (inorganic phosphate was accumulated in the glycolytic intermediate metabolite pool), which would contradict both experimental results and the model of oxidative phosphorylation. It was checked that these changes did not affect significantly the kinetic properties of glycolysis, especially the phenomenological glycolytic flux/ADP relationship (see below). The discussed joined model was used in the simulations presented in Figs. 2, 4 and 5.

However, because the model of glycolysis developed by Lambeth and Kushmerick appeared to be unable to account for the kinetic properties of glycolysis in skeletal muscle (see below), a simple kinetic description of the dependence of the glycolytic ATP and H^+ production on [ADP] was extracted from model, and this description was supplemented with the direct activation of glycolysis during muscle contraction and with the inhibition of this metabolic pathway by H^+ ions.

The main external metabolites affecting the glycolytic flux under physiological conditions are ADP, AMP and P_i [3,14] (glycolysis is very sensitive to ATP [14], but the concentration of this metabolite is essentially constant in muscle except during extremely intensive exercise where anaerobic glycolysis is the main source of ATP). The relation between the concentration of ADP, AMP, ATP and P_i is unique at different energetic states (energy demands) as long as the concentration of the adenine nucleotide pool (ATP+ADP+AMP) remains constant and creatine ki-

nase and adenylate kinase are not significantly displaced from thermodynamic equilibrium. These two conditions may be not fulfilled during short-term extremely intensive exercise, where AMP is converted to IMP (and thus the size of the adenine nucleotide pool decreases) and creatine kinase may be displaced from equilibrium. However, in the present article only the exercise intensities at which oxidative phosphorylation is still the main source of ATP are considered; these intensities correspond to less than a half of the maximal ATP usage in muscle, considering the maximal capacity of oxidative phosphorylation and anaerobic glycolysis [1,2].

For these reasons, the kinetic dependence of the glycolytic flux on ADP, AMP, ATP and P_i was described in the present article as a phenomenological relationship between the rate of glycolysis and [ADP], including implicitly the dependence on [AMP], [ATP] and [P_i]. ADP was chosen as the representative of the adenine nucleotide and phosphate pool, because glycolysis is very sensitive to [ADP] and at the same time [ADP] changes significantly during transition from rest to work [14]. The phenomenological dependence of the glycolytic flux on ADP concentration was extracted by running the joined model (the model of glycolysis developed by Lambeth and Kushmerick incorporated into the model of oxidative phosphorylation, as described above), setting different rate constants of ATP usage and recording the values of glycolytic flux and [ADP]. The simulations were performed under the assumption that 40% of GP (glycogen phosphorylase) appears in the form A, as in the original article [14]. The results of these simulations are presented in Fig. 2. A quite similar phenomenological glycolytic flux/ADP relationship was obtained using the original model of 'isolated' glycolysis [14] [not shown].

Because the regulation of glycolysis by ADP (plus AMP and P_i) appeared to be decidedly insufficient to cause the great increase in the glycolytic flux observed in intact skeletal muscle, and therefore a strong direct activation of glycolysis had to be involved (see below), the dependence presented in Fig. 2 was

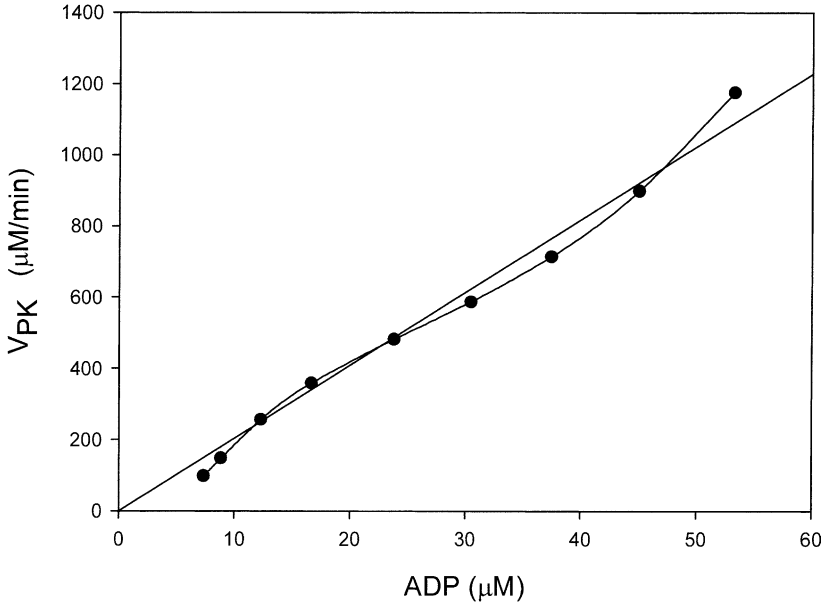

Fig. 2. Simulated phenomenological dependence between the glycolytic flux and ADP concentration. This dependence involves implicitly the dependence on [AMP], [ATP] and [P_i]. The simulated points (black circles) are fitted by a simple linear equation (straight line). Simulations were made using the model of glycogenolysis and glycolysis in skeletal muscle developed by Lambeth and Kushmerick [14] incorporated into the model of oxidative phosphorylation in skeletal muscle developed by Korzeniewski et al. [5,11].

roughly approximated by a simple linear dependence (see Fig. 2):

$$v_{GLYC} = k_{GLYC} \cdot ADP_{te} \quad (1)$$

where ADP_{te} is the total (magnesium-bound and magnesium-free) free cytosolic ADP concentration, v_{GLYC} is the rate of the glycolytic pyruvate/lactate/H^+ production, while the rate constant k_{GLYC} is calculated under the assumption that in resting state the lactate/pyruvate production by glycolysis is exactly equal to the lactate/pyruvate consumption by oxidative phosphorylation (it is assumed that in resting state the rate of glycolysis is equal to 0.2 of the substrate dehydrogenation rate v_{DH}, since five NADH/$FADH_2$ molecules are produced by pyruvate dehydrogenase and tricarboxylate acid (TCA) cycle per each pyruvate molecule).

The simulated curve presented in Fig. 2 can be reproduced much more exactly using a polynomial fit instead of a simple linear fit. However, such a polynomial description does not change significantly the general predictions formulated in the present article [data not shown], while at the same time it is much more complicated and unclear. For this reason we incorporated the linear description into the model of oxidative phosphorylation and used the joined model in the simulations presented in Figs. 6 and 7.

The above description is roughly equivalent to the dependence of the glycolytic flux on ADP, AMP, ATP and P_i contained explicitly in the model of glycolysis developed by Lambeth and Kushmerick [14], and its use is fully justified if somebody is not interested what happens inside the glycolytic system. Such a description has also the advantage that a simple dependence of glycolysis on proton concentration and the direct activation of glycolysis can be easily incorporated to this description—it would be much more difficult to do this with the original model by Lambeth and Kushmerick, where the dependence on pH and direct activation of each particular enzyme would have to be included. The available experimental data are simply insufficient to decide which enzymes are directly activated during muscle contraction and inhibited by protons, and to what extent.

It is a well known fact that the glycolytic flux is inhibited by H^+ ions [3]. For example, in the model of glycolysis in red blood cells developed by Mulquiney and Kuchel [15] the rates of the reactions catalyzed by four enzymes (HK, PFK, GADPH and PK) depend on proton concentration. For instance, a decrease of pH by only 0.2 pH units inhibits phosphofructokinase (PFK) by more than 10 times [not shown]. However, the regulation of glycolysis in red cells is likely to be different from the regulation of this process in skeletal muscle. Therefore, the message coming from the discussed model for red cells is that glycolysis in muscle is probably sensitive to H^+, but the exact quantitative dependence of the glycolytic flux in this tissue on the proton concentration is not known.

Therefore, a simple linear description of the dependence of glycolysis on H^+ is used in the second version of the model used in the present work (simulations presented in Figs. 8 and 9):

$$v_{GLYC} = k_{GLYC} \cdot ADP_{te}\left(H^+_{rest}/H^+\right) \quad (2)$$

where $H^+_{rest}=10^{-7}$ M (pH 7.0) is the resting proton concentration.

In the present work it was assumed that glycolysis is activated $n^{0.55}$ times in the absence of the inhibition by protons (Eq. (1), Figs. 6 and 7) and $n^{0.8}$ times in the presence of the inhibition by protons (Eq. (2), Figs. 8 and 9), in parallel with an $n^{0.4}$-fold activation of oxidative phosphorylation and n-fold activation of ATP usage during rest-to-work transition, in order to cause a decrease in pH by 0.1–0.2 pH unit during moderate exercise and by 0.5–0.6 unit during intensive exercise. The direct activation of ATP usage, oxidative phosphorylation and glycolysis caused by some contraction-related intracellular factors(s) (e.g. calcium ions) transducing the signal from neural stimulation and not being an intermediate metabolite is equivalent to an increase of the rate constants of these processes.

Protons can potentially inhibit the ATP-usage system because H^+ is one of the products of the ATP hydrolysis reaction. However, a constant power output during muscle work is assumed in the computer simulations presented in the present article. Therefore, if any inhibition of ATP consumption by protons takes

place, it must be compensated in some way, for instance by a more intensive neural stimulation of muscle.

In the computer model of oxidative phosphorylation with a simple kinetic description of glycolysis used in the present article, the rate of glycolytic ATP production appears in the differential equations describing changes over time of cytosolic [ATP], [ADP] and [P_i]:

$$\dot{\mathrm{ATP}}_{te} = v_{EX} - v_{UT} + v_{CK} + v_{AK} + 1.5\, v_{GLYC} \quad (3)$$

$$\dot{\mathrm{ADP}}_{te} = v_{UT} - v_{EX} - v_{CK} - 2\, v_{AK} - 1.5\, v_{GLYC} \quad (4)$$

$$\dot{\mathrm{Pi}}_{te} = v_{UT} - v_{PI} - 1.5\, v_{GLYC} \quad (5)$$

where v_X, is the rate of reaction/process X (EX, ATP/ADP carrier; PI, phosphate carrier; UT, ATP usage; CK, creatine kinase; AK, adenylate kinase; GLYC, glycolysis), while the rate of glycolytic H^+ production appears in the differential equation describing changes in time of cytosolic [H^+]:

$$\dot{H}_e^+ = \begin{pmatrix} 2(2+2u)v_{C4} + (4-2u)v_{C3} + 4v_{C1} - n_A v_{SN} - u v_{EX} \\ -(1-u)v_{PI} - v_{LK} - v_{CK} - v_{EFF} + v_{GLYC} - 0.2 v_{DH} \end{pmatrix} / r_{buffe} \quad (6)$$

The complete description of the computer model of oxidative phosphorylation+glycolysis in skeletal muscle used in the present study is located at the web site: http://www.mol.uj.edu.pl/~benio.

2.2. Computer simulations

Computer simulations presented in the present article were performed either using the model of oxidative phosphorylation plus comprehensive model of glycolysis developed by Lambeth and Kushmerick (Figs. 2, 4 and 5) (these mode of simulations will be called Mode 0) or the model of oxidative phosphorylation containing the simple kinetic description of the glycolytic flux as a function of ADP concentration (Eq. (1) or Eq. (2)). In the latter case, three further modes of simulations were used in the simulations performed in the present paper. In Mode 1, no ATP and H^+ production by anaerobic glycolysis is involved (it is assumed that the pyruvate/lactate/proton production by glycolysis is always equal to the pyruvate/lactate/proton consumption by oxidative phosphorylation) (Fig. 3). In Mode 2, only the dependence of the glycolytic flux on ADP (Eq. (1)) and the direct activation of glycolysis during muscle contraction are included. In Mode 3, the glycolytic flux is described as depending on both ADP and H^+ (Eq. (2)), and is also directly activated during muscle contraction. Thus, the production of ATP and H^+ by anaerobic glycolysis is allowed in Mode 2 and Mode 3.

During transition from rest to work, it was assumed that ATP demand (rate constant of ATP usage) increased either 30 times (moderate exercise) or 100 times (intensive exercise, where oxidative phosphorylation is still the main source of ATP). It was also assumed that oxidative phosphorylation is directly activated by some cytosolic factor (e.g. calcium ions), in parallel with the activation of ATP usage by calcium ions [12,16,17]. Therefore, the rate constants of all oxidative phosphorylation steps but proton leak were increased $n^{0.4}$ times, together with the n (30 or 100)-fold increase in the rate constant of ATP usage. It must be emphasized that the relative increase in the respiration rate in active state in relation to resting state is over twice smaller than the relative increase in energy demand and ATP turnover. For example, a 30-fold and 100-fold increase in energy demand leads to an approximately 10–13-fold and 35–40-fold increase in V_{O_2}, respectively, (see Figs. 3–8). This is because the proton leak accounts for over 50% of oxygen consumption in resting state (the remaining less than 50% being due to ATP synthesis [18]) and because some fraction of ATP is delivered from anaerobic glycolysis and not from oxidative phosphorylation. Finally, in Mode 2 glycolysis was directly activated $n^{0.55}$ times during muscle contraction, while in Mode 3 glycolysis was activated $n^{0.8}$ times in order to compensate the inhibition of glycolysis by protons.

3. Theoretical results and discussion

3.1. No anaerobic glycolysis

First, in order to create a reference point for other simulations and to investigate the role and regulation

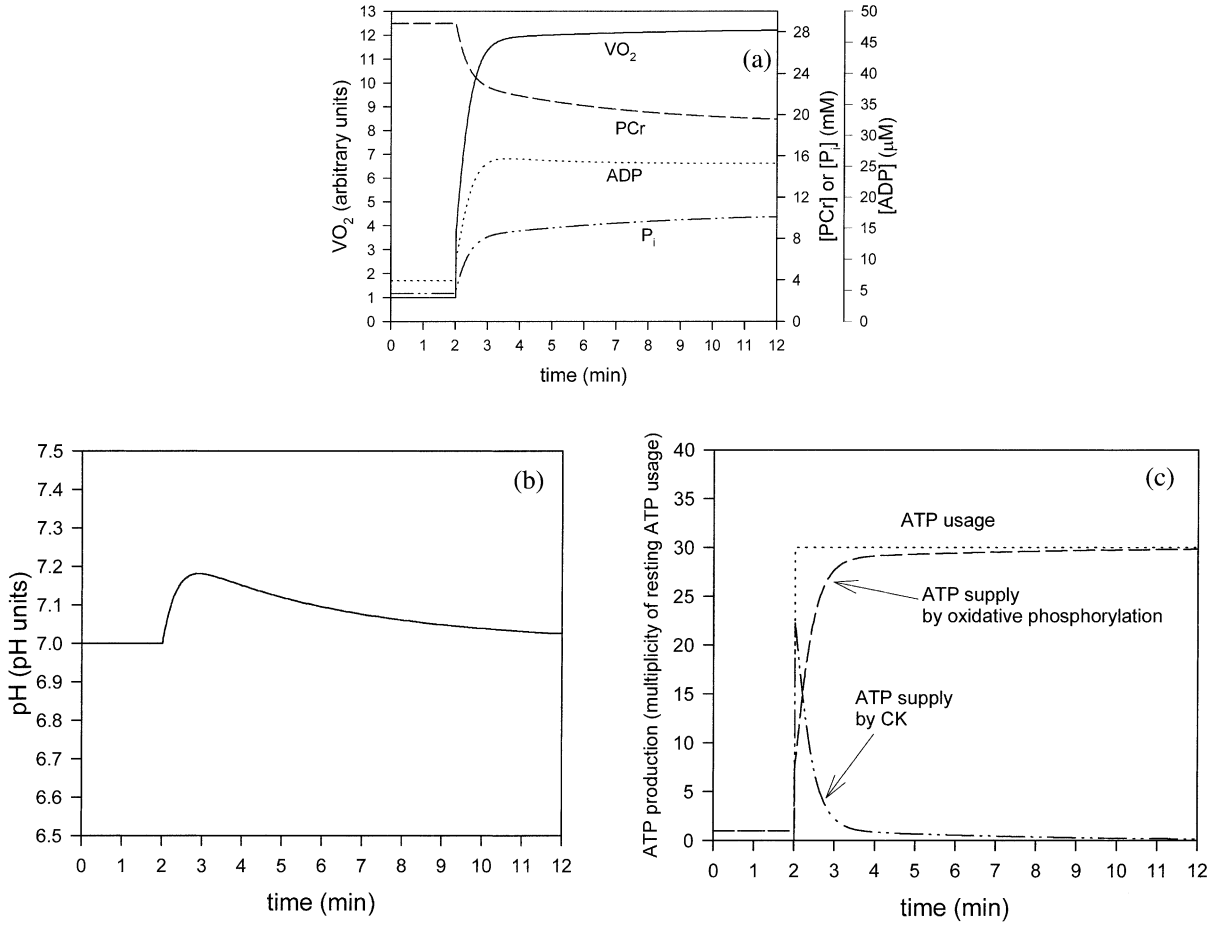

Fig. 3. Simulated behavior of the bioenergetic system in muscle in Mode 1 during transition from rest to moderate exercise. Mode 1: no anaerobic glycolysis, pyruvate consumption by oxidative phosphorylation (+TCA cycle) exactly matches pyruvate production by glycolysis. After first 2 min of simulation, representing resting steady-state, ATP usage was increased 30 times. In the same moment oxidative phosphorylation was activated $30^{0.4}$ times. (a) time courses of $\dot{V}O_2$ (standardized for 1 in resting state), ADP, PCr and P_i; (b), time course of cytosolic pH; (c) time courses of ATP usage, ATP supply by creatine kinase and ATP supply by oxidative phosphorylation (including aerobic glycolysis).

of anaerobic glycolysis in a muscle cell, a system without anaerobic glycolysis was simulated. This is Mode 1 described above. It was assumed in these simulations that the consumption of pyruvate by oxidative phosphorylation matches exactly the pyruvate production by glycolysis. Therefore, there takes place no net glycolytic lactate and H^+ production, while the glycolytic ATP supply is equal to 0.3 (0.2×1.5) of the rate of substrate dehydrogenation (five $NADH/FADH_2$ molecules are produced by pyruvate dehydrogenase and TCA cycle per each pyruvate molecule produced by glycolysis and 1.5 ATP molecules are produced per each pyruvate molecule in glycolysis from glycogen).

Fig. 3a presents the simulated changes over time of the respiration rate ($\dot{V}O_2$), ADP, PCr and P_i in Mode 1 during transition from rest to work, after an onset of moderate (30-fold increase in ATP usage) exercise. It can be seen that the respiration rate (scaled to equal 1 in rest) increases quickly (within 1 min) approximately 12 times after an onset of moderate exercise (in the result of direct activation and changes in [ADP] and [P_i]) and

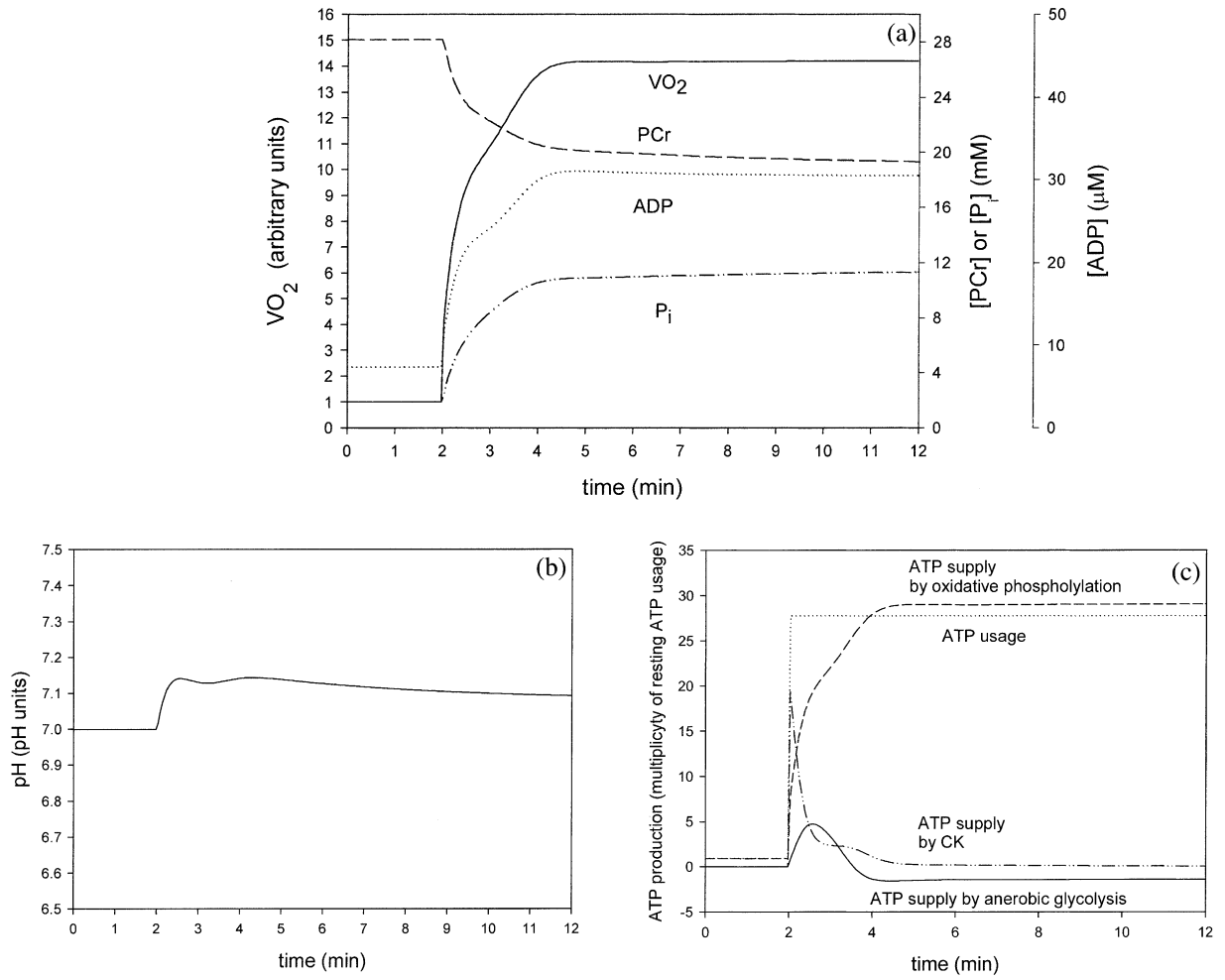

Fig. 4. Simulated behavior of the bioenergetic system in muscle in Mode 0 during transition from rest to moderate exercise. Mode 0: activation of glycolysis by ADP, AMP and P_i within the model of glycolysis developed by Lambeth and Kushmerick [14]. After first 2 min of simulation, representing resting steady-state, ATP usage was increased 30 times. In the same moment oxidative phosphorylation was activated $30^{0.4}$ times. (a) time courses of $\dot{V}O_2$ (standardized for 1 in resting state), ADP, PCr and P_i; (b), time course of cytosolic pH; (c), time courses of ATP usage, ATP supply by creatine kinase, ATP supply by anaerobic glycolysis and ATP supply by oxidative phosphorylation (including aerobic glycolysis).

then stabilizes at a steady-state value. [ADP] also increases quickly from 7 to 25 to μM and then remains almost constant. At the same time, the concentration of phosphocreatine first decreases quickly and then continues to decrease with a much smaller rate. A similar, although oppositely-directed behavior characterizes the time course of the concentration of inorganic phosphate—after an initial rapid increase there appears a phase with a much slower continuous increase.

This non-steady-state behavior of [PCr] and [P_i] is caused by changes in H^+, affecting the equilibrium of creatine kinase. Fig. 3b presents the simulated time course of cytosolic pH after an onset of exercise. It can be seen that pH first increases by approximately 0.2 pH units and then slowly decreases, approaching asymptotically the resting pH value (7.0). The initial alkalization is caused by the consumption of protons by creatine kinase, while

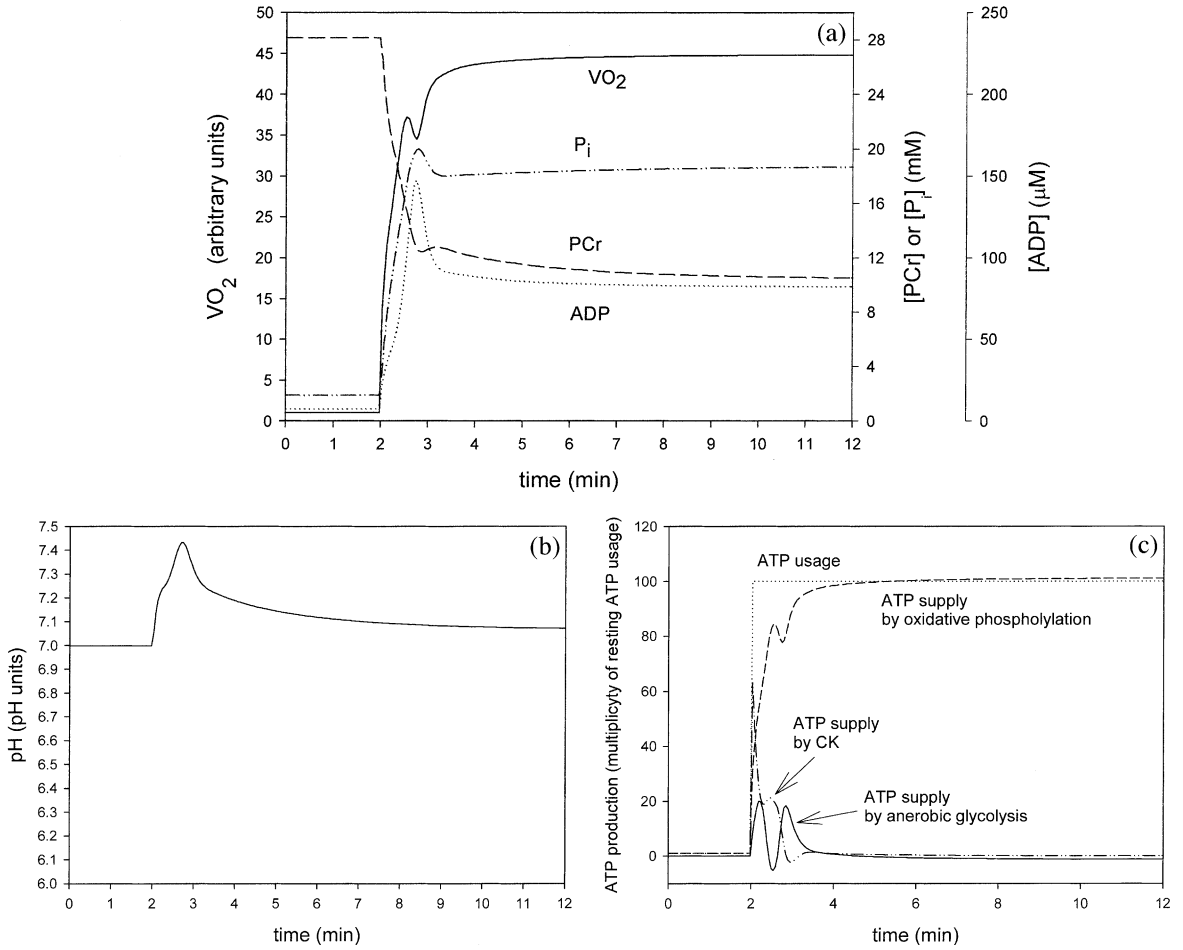

Fig. 5. Simulated behavior of the bioenergetic system in muscle in Mode 0 during transition from rest to intensive exercise. Mode 0: activation of glycolysis by ADP, AMP and P_i within the model of glycolysis developed by Lambeth and Kushmerick [14]. After first 2 min of simulation, representing resting steady-state, ATP usage was increased 100 times. In the same moment oxidative phosphorylation was activated $100^{0.4}$ times. (a) time courses of V_{O_2} (standardized for 1 in resting state), ADP, PCr and P_i; (b) time course of cytosolic pH; (c) time courses of ATP usage, ATP supply by creatine kinase, ATP supply by anaerobic glycolysis and ATP supply by oxidative phosphorylation (including aerobic glycolysis).

the return to the resting proton concentration that occurs afterwards is caused by the influx of protons from blood, postulated previously [5]. Of course, no acidification of cytosol below the resting pH value takes place, because in Mode 1 no net proton production by anaerobic glycolysis is involved. The initial alkalization predicted in the above simulations performed in Mode 1 is much greater and longer than the initial alkalization observed in experimental studies—in the latter case the maximal extent of an increase in pH is approximately 0.1 pH units and the whole alkalization phase lasts approximately 0.5–1.5 min [8].

Fig. 3c presents the simulated changes over time of ATP usage and of particular reactions contributing to ATP production. One can see that in the absence of anaerobic glycolysis, in the initial phase of exercise most of ATP is supplied from the reaction catalyzed by creatine kinase (conversion of PCr to Cr), but after a short time (0.5–1 min) almost entire ATP supply is taken over by oxidative phosphorylation (including aerobic glycolysis).

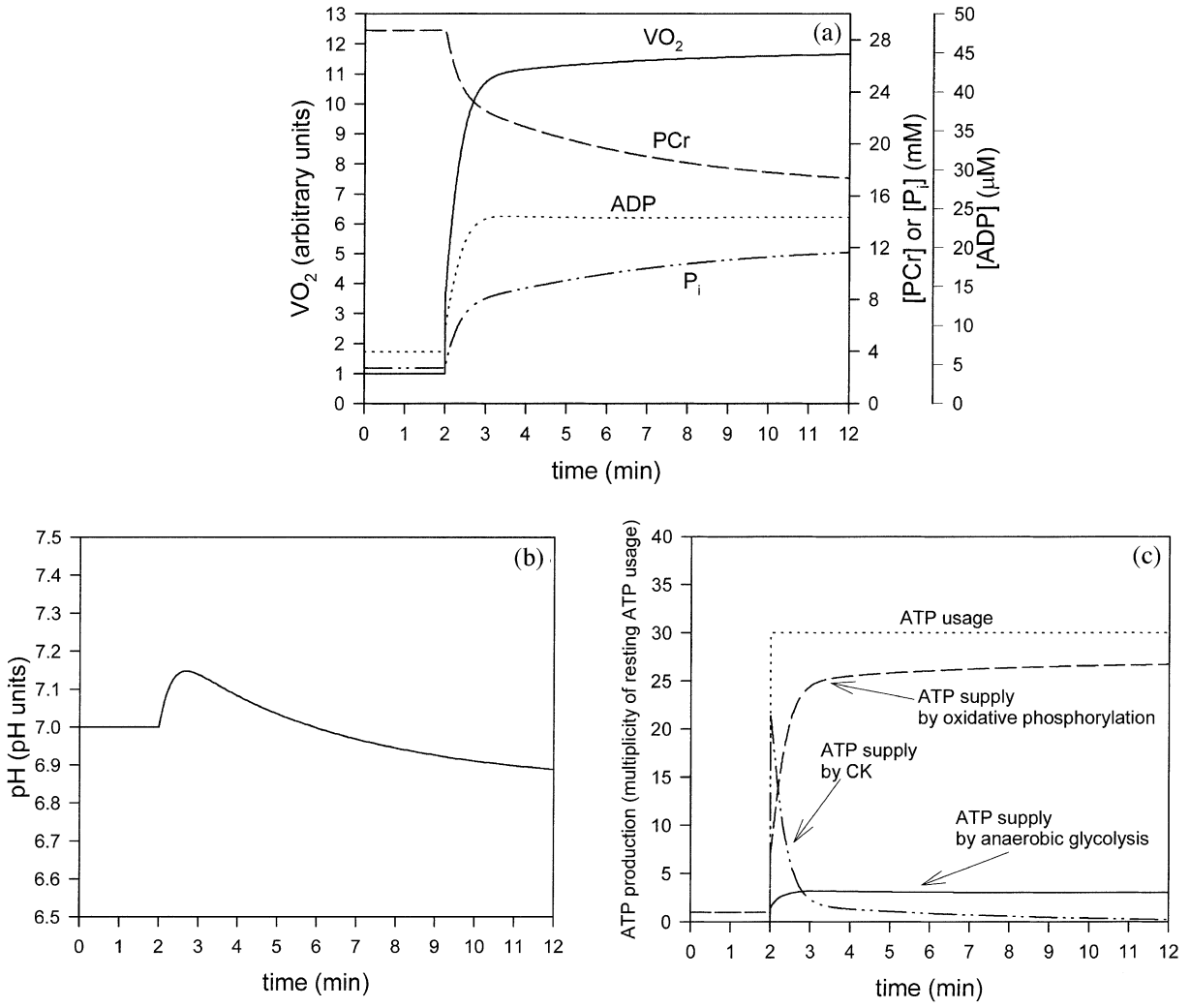

Fig. 6. Simulated behavior of the bioenergetic system in muscle in Mode 2 during transition from rest to moderate exercise. Mode 2: direct activation of glycolysis during muscle contraction, activation of glycolysis by ADP (plus AMP and P_i). After first 2 min of simulation, representing resting steady-state, ATP usage was increased 30 times. In the same moment, glycolysis was directly activated $30^{0.55}$ times and oxidative phosphorylation was activated $30^{0.4}$ times. (a) time courses of V_{O_2} (standardized for 1 in resting state), ADP, PCr and P_i; (b) time course of cytosolic pH; (c) time courses of ATP usage, ATP supply by creatine kinase, ATP supply by anaerobic glycolysis and ATP supply by oxidative phosphorylation (including aerobic glycolysis).

As it was discussed previously [5], in the absence of H^+ production by anaerobic glycolysis the initial alkalization of the cytosol in skeletal muscle after an onset of high-intensity exercise may lead to an instability of the system. This instability is caused by a sort of a positive feedback, in which the initial decrease in ATP/ADP, caused by an increase in ATP usage, leads to proton consumption by CK, to a shift in the equilibrium of this enzyme and thus to a further decrease in ATP/ADP [5]. An additional factor (not considered explicitly in the cited article) contributing to the discussed instability is the shift in the equilibrium between P_i^- and P_i^{-2} caused by alkalization, in the direction of the latter form (P_i^{-2}) production. Because only the former form (P_i^-) is transported by the phosphate carrier through the

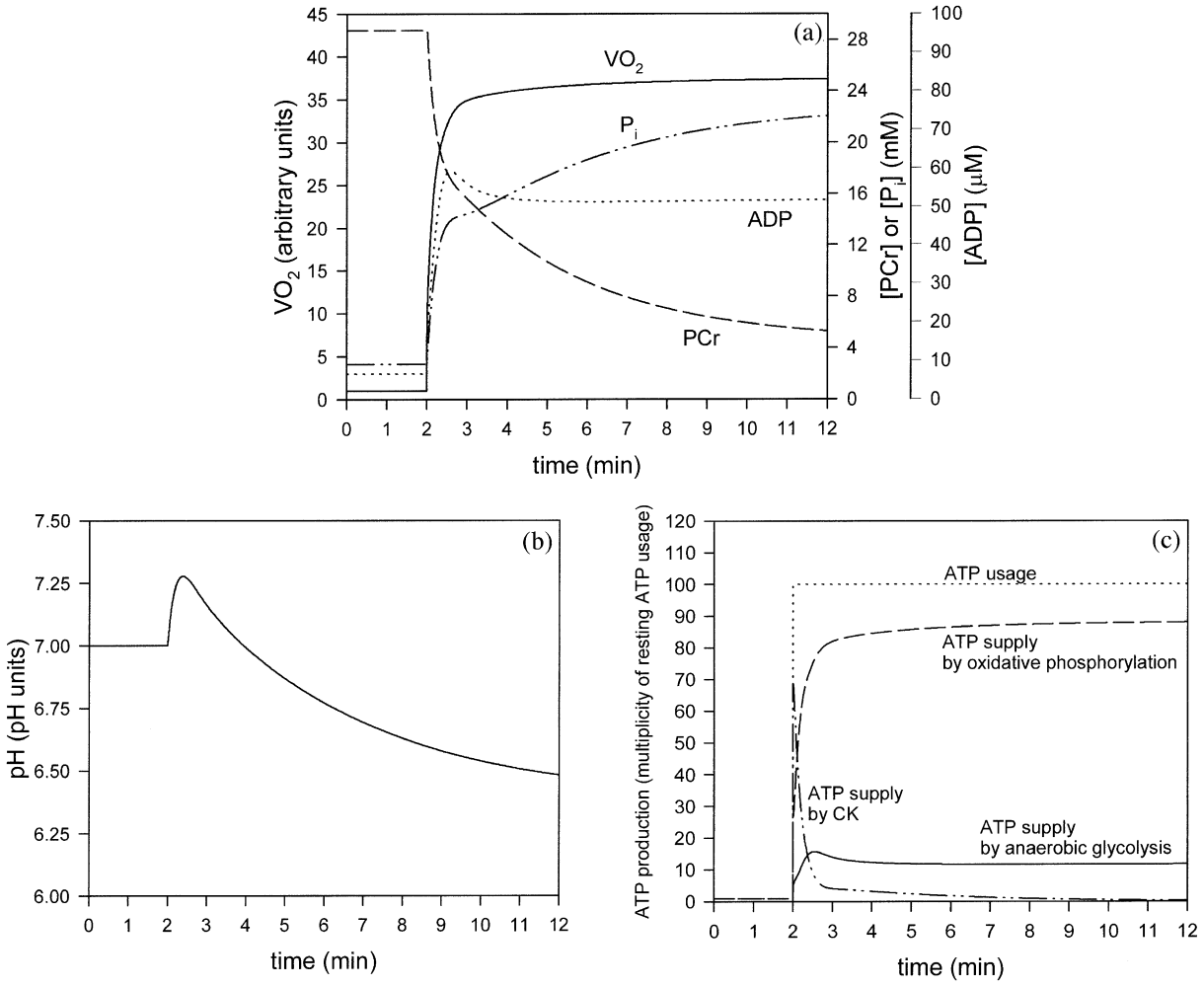

Fig. 7. Simulated behavior of the bioenergetic system in muscle in Mode 2 during transition from rest to intensive exercise. Mode 2: direct activation of glycolysis during muscle contraction, activation of glycolysis by ADP (plus AMP and P_i). After first 2 min of simulation, representing resting steady-state, ATP usage was increased 100 times. In the same moment, glycolysis was directly activated $100^{0.55}$ times and oxidative phosphorylation was activated $100^{0.4}$ times. (a) time courses of $\dot{V}O_2$ (standardized for 1 in resting state), ADP, PCr and P_i; (b) time course of cytosolic pH; (c) time courses of ATP usage, ATP supply by creatine kinase, ATP supply by anaerobic glycolysis and ATP supply by oxidative phosphorylation (including aerobic glycolysis).

inner mitochondrial membrane, the discussed effect of an increase in pH slows down the transport of P_i inside mitochondria, what can lead to a substantial decrease in the mitochondrial phosphate concentration and to inhibition of respiration and ATP production [not shown]. This effect leads to a further decrease in ATP/ADP and may contribute to the discussed instability of the system. It must be stressed that this instability would appear at much lower work intensities in the absence of the postulated proton influx and of the parallel activation in ATP supply–demand system proposed previously [12,16,17].

Because the instable behavior of the system is not strictly relevant for the topic of the present work, and because it was presented previously [5], the simula-

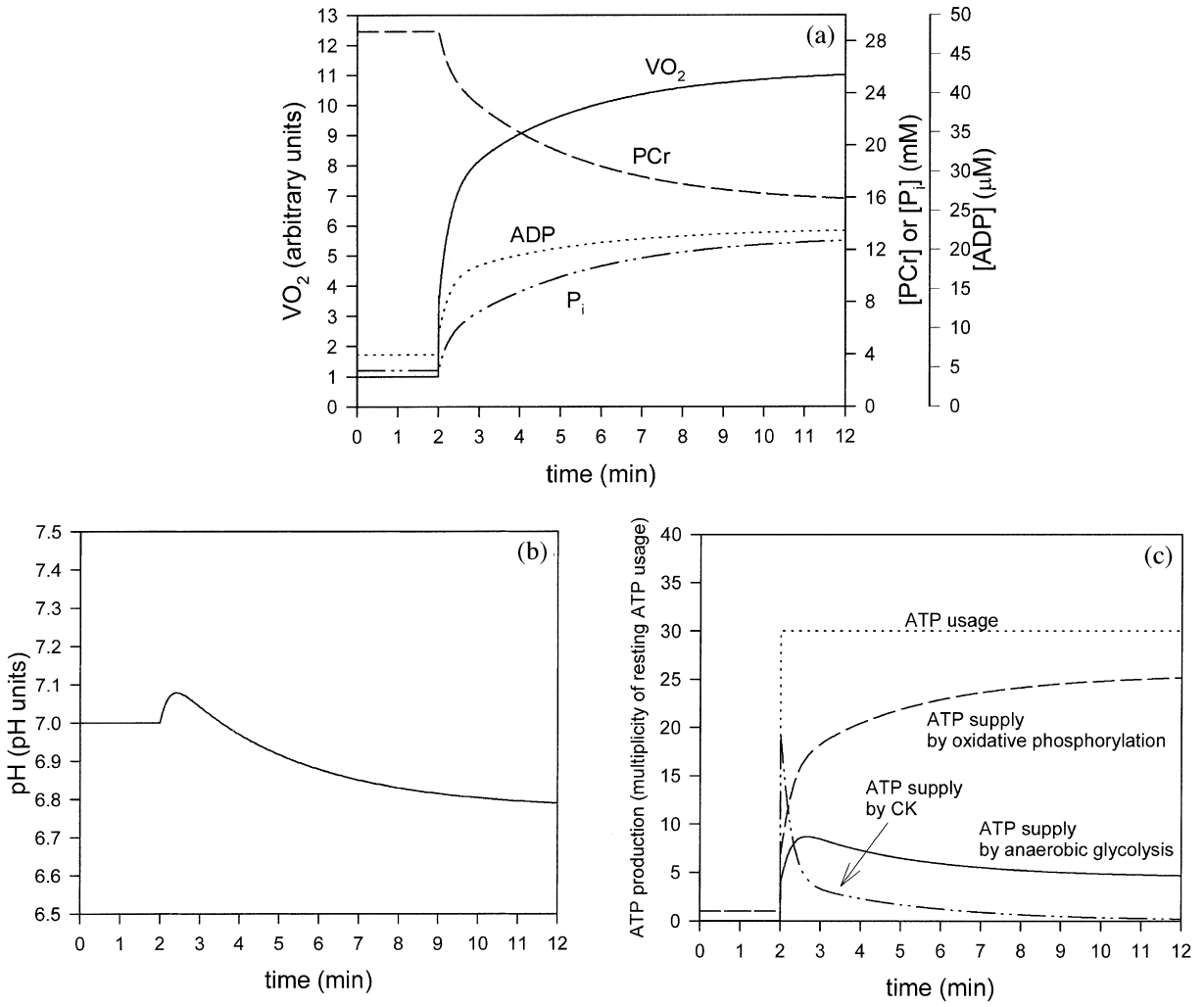

Fig. 8. Simulated behavior of the bioenergetic system in muscle in Mode 3 during transition from rest to moderate exercise. Mode 3: direct activation of glycolysis during muscle contraction, activation of glycolysis by ADP (plus AMP and P_i), inhibition of glycolysis by H^+. After first 2 min of simulation, representing resting steady-state, ATP usage was increased 30 times. In the same moment, glycolysis was directly activated $30^{0.8}$ times and oxidative phosphorylation was activated $30^{0.4}$ times. (a) time courses of V_{O_2} (standardized for 1 in resting state), ADP, PCr and P_i; (b) time course of cytosolic pH; (c) time courses of ATP usage, ATP supply by creatine kinase, ATP supply by anaerobic glycolysis and ATP supply by oxidative phosphorylation (including aerobic glycolysis).

tions concerning the intensive (100-fold increase in ATP usage) exercise in Mode 1 are not presented in the present article.

3.2. Dependence of glycolytic flux on ADP, AMP, ATP and P_i

Figs. 4 and 5 present changes over time of selected parameter values simulated using the model of oxidative phosphorylation containing the original model of glycolysis developed by Lambeth and Kushmerick (Mode 0), for a transition from rest to moderate and intensive exercise, respectively. The most characteristic property of the simulations presented in Fig. 4, shown in Fig. 4c, is that the flux of anaerobic glycolysis becomes negative here. The meaning of the discussed theoretical result is that glycolysis is relatively little activated by changes in

ADP, AMP and P_i occurring during rest-to-work transition. This activation is insufficient not only to cause an additional ATP (and H^+) supply by anaerobic glycolysis, but also to match the increased pyruvate consumption during muscle work. Of course, such a situation is possible only in computer simulations in which glycolysis is linked kinetically with oxidative phosphorylation via ADP, AMP and P_i, but not via pyruvate. In the real system, or in a complete model including also TCA cycle, the shortage of substrate (pyruvate) would block oxidative phosphorylation and cause a great, unphysiological increase in ADP and inorganic phosphate concentration. The persistent alkalization of cytosol during exercise presented in Fig. 4b is of course an artifact caused by the consumption of protons by 'negative anaerobic glycolysis'. The simulated glycolytic metabolite concentrations change after the onset of exercise by a few orders of magnitude [not shown], what contradicts experimental results [7] (these changes are responsible for the decrease in the glycolytic ATP supply after the initial activation seen in Fig. 4c).

It can be deduced from Fig. 5, presenting simulations performed using the model in Mode 0 for transition from rest to intensive exercise, that in this case the intensity of the glycolytic flux is too small to fully prevent the instability of the system after the onset of exercise discussed above and in Ref. [5]. Additionally, in this case in the active steady-state, the production of pyruvate by glycolysis is also lower than consumption of pyruvate by oxidative phosphorylation (plus TCA cycle) and therefore we deal with 'negative' anaerobic glycolysis. As it was discussed above, the negative anaerobic glycolysis has no real meaning and serves only to show that the model of glycolysis developed by Lambeth and Kushmerick, if not supplemented with additional regulatory mechanisms, leads to a paradox.

The reason for the behavior described above may be deduced from Fig. 2. One can see that an increase in the ADP concentration from 7 to over 50 μM (and appropriate changes in [AMP] and $[P_i]$), which roughly corresponds the maximal change in this parameter during transition from rest to intensive (but not maximal) exercise, is able to stimulate the glycolytic flux only approximately 10 times, while an at least 100-fold increase in the glycolytic flux is encountered in the real system. Even if no anaerobic glycolysis is taken into account, over 35-fold increase in the glycolytic flux is needed in order to meet the increased respiration rate (and pyruvate consumption) at intensive exercise (see Fig. 5). Additionally, the simulated half-transition time from resting glycolytic flux to active glycolytic flux was very long here, longer than 3 min [not shown], what clearly contradict the quick activation of glycolysis encountered in skeletal muscle [3]. Finally, as it was mentioned above, simulations performed in Mode 0 predict very large relative changes in glycolytic metabolite concentrations, what is not encountered in experimental studies [7]. Therefore, the activation of the glycolytic flux (involving the activation of the 'key' glycolytic enzymes—glycogen phosphorylase and phosphofructokinase) by the increase in [ADP], [AMP] and $[P_i]$, taken into account within the model of glycolysis developed by Lambeth and Kushmerick, seems to be decidedly insufficient to explain alone the behavior of the glycolytic pathway in intact skeletal muscle.

3.3. Effect of direct activation of glycolysis

In order to test the effect of the postulated direct activation of glycolysis during muscle contraction on the kinetic behavior of the system, computer simulations in Mode 2 (simple dependence of glycolysis on ADP and, implicitly, on AMP, ATP and P_i, extracted from the model developed by Lambeth and Kushmerick plus direct activation of glycolysis at the onset of exercise) were performed. Fig. 6 presents the results of these simulations for transition from rest to moderate exercise. It can be seen in Fig. 6a that the increase in the respiration rate is slightly lower here than in Mode 1. This is because the activated glycolysis takes over some fraction of ATP production from oxidative phosphorylation. The 'slow' phase in the time course of PCr and P_i is slightly faster in Mode 2 than in Mode 1. This is caused by continuously proceeding acidification of cytosol caused by proton production by anaerobic glycolysis (see below); this acidification shifts in turn the equilibrium of creatine kinase.

Fig. 6b presents the simulated changes in cytosolic pH after an onset of moderate-intensity exercise in Mode 2. The initial alkalization is smaller here (up to approx. 0.15 pH units) and lasts shorter (approx. 4 min) than in Mode 1. Therefore, in this aspect, Mode 2 seems to match the experimental results [8] better than Mode 1. During the acidification phase that follows the alkalization phase in Mode 2, pH drops by over 0.1 pH units in relation to resting pH, which agrees well with the decrease in pH (by 0.1–0.2 pH units) measured experimentally during moderate-intensity exercise [8–10].

Fig. 6c presents the simulated time course of ATP usage and of ATP supply by three processes: CK-catalyzed reaction, anaerobic glycolysis and oxidative phosphorylation (including aerobic glycolysis). It can be seen that in the working quasi-steady-state the anaerobic glycolysis takes over approximately 10% of ATP production from oxidative phosphorylation. In the discussed simulation a sustained activation of anaerobic glycolysis takes place after an onset of exercise, while only a transient stimulation of anaerobic glycolysis is encountered in experimental studies [3,4].

In Fig. 7 there is shown a simulation analogous to that presented in Fig. 6 (also concerning Mode 2), but for an exercise of high intensity (100-fold increase in ATP usage). The first conclusion that can be drawn is that H^+ supply by anaerobic glycolysis is able to prevent in this case the potential instability of the system discussed above and in Ref. [5]. In the presented simulation, V_{O_2} increases approximately 35 times (Fig. 7a) during rest→work transition. However, this increase could be essentially greater if the glycolytic ATP supply was not greatly stimulated (Fig. 7c). In this simulation [PCr] decreases, while [P_i] increases continuously and no active steady-state is achieved. Such a behavior is caused by continuously proceeding production of protons by anaerobic glycolysis and acidification of cytosol (Fig. 7b), which affects the equilibrium of creatine kinase. The extent of the initial alkalization is great here, reaching maximally 0.25 pH units. The glycolytic flux increases significantly after the onset of exercise and, after a small overshoot, stabilizes at constant working value corresponding to approximately 12% of ATP usage during exercise.

The simulations performed in Mode 2 and presented in Figs. 6 and 7 demonstrate semi-quantitatively that direct activation of glycolysis during muscle contraction is able, at least in principle, to elevate the rate of ATP, pyruvate and H^+ production by glycolysis to the level encountered in the real system during moderate and intensive exercise, at which oxidative phosphorylation is still the main source of ATP in the active (quasi-) steady-state. Nevertheless, there is still some discrepancy between these simulations and experimental results. First, the simulated extent and duration of the initial alkalization is much greater than that encountered in experimental studies, where initial alkalization reaches up to 0.1 pH units and lasts 0.5–1.5 min [3,8]. Second, a sustained activation of glycolysis takes place, while only a transient stimulation of glycolytic flux after the onset of exercise is encountered in experiments, at least at high work intensities [3]. Therefore, it is likely that the regulation of glycolysis by ADP (plus AMP and P_i) and by direct activation during exercise is still insufficient to explain the experimental results and that some additional regulatory factor is needed.

3.4. Effect of inhibition of glycolysis by H^+ ions

This lacking factor may be the inhibition of glycolysis by protons [[3,15], see also above]. Fig. 8 presents the results of the simulation of transition from resting state to moderate exercise (30-fold increase in ATP usage) in Mode 3 (glycolysis dependent on adenine nucleotide and inorganic phosphate concentration as well as on proton concentration, glycolysis directly activated during muscle contraction). It can be seen (Fig. 8a) that [PCr] and [P_i] slowly approach to steady-state values, which is associated with a slow approach of proton concentration to a steady-state (Fig. 8b). The time courses and active-steady-state values of the concentrations of these metabolites match well the experimental data concerning moderate exercise [8,9]. The time course of cytosolic pH after an onset of moderate-intensity exercise is presented in Fig. 8b. After an initial alkalization, reaching maximally 0.1 pH units and lasting approximately 1.5 min, there follows a phase of acidification, in which pH approaches asymptotically to the value

of approximately 6.8 (approx. 0.2 pH units below the resting pH equal to 7.0). The extent of acidification predicted theoretically within Mode 3 agrees well with experimental results, where pH drops by 0.1–0.2 pH units during moderate exercise [3,8–10]. The extent and duration of the initial alkalization also reflects well experimental data [8]. Fig. 8b presents the simulated time courses of ATP usage and of ATP production by oxidative phosphorylation, creatine kinase and anaerobic glycolysis. One can see that, after an initial activation of anaerobic glycolysis after an onset of exercise, the intensity of this process decreases significantly, tending to some steady-state. The peak contribution of anaerobic glycolysis to the overall ATP production reaches 29%, while in the active steady-state, this contribution drops to approximately 16%. The glycolytic flux is first activated by a direct activation, by an increase in [ADP] (plus [AMP] and [P_i]) and by the initial alkalization, and then it is inhibited by the advancing acidification. This transient activation of anaerobic glycolysis after an onset of exercise agrees well (although only semi-quantitatively) with the widely-accepted opinion in the literature concerning the behavior of the bioenergetic system in muscle [3,4].

Generally, it can be concluded that Mode 3 (dependence of glycolysis on ADP and H^+) gives predictions much better matching experimental results than the predictions produced within Mode 2 (dependence of glycolysis on ADP only). Namely, the simulations in Mode 3 predict the transient activation of anaerobic glycolysis and the proper extent and duration of the initial alkalization.

In order to reveal the effect of anaerobic glycolysis on the oxidative phosphorylation system in skeletal muscle it is necessary to compare Mode 3 (proper semi-quantitative kinetic description of glycolysis) with Mode 1 (no anaerobic glycolysis involved). From the analysis of Figs. 3 and 8 it can be concluded that anaerobic glycolysis significantly slows down the transition of V_{O_2} between 0.5 and 3 min after an onset of exercise. This is caused, of course, by a transient activation of ATP supply by anaerobic glycolysis. However, the time course of V_{O_2} during the first 30 s of exercise is little affected, and therefore the presence of anaerobic glycolysis does not lengthen much the transition time ($t_{1/2}$).

Although anaerobic glycolysis slows down V_{O_2} transition, the transition of [PCr], [ADP] and [P_i] between rest and exercise is not essentially delayed. Such a behavior is caused by the presence of an additional source of ATP supply, namely anaerobic glycolysis. Of course, in the presence of anaerobic glycolysis the concentrations of the mentioned metabolites stabilize at different levels in the active steady state, comparing with the system without anaerobic glycolysis, since the lowered cytosolic pH shifts the equilibrium of the reaction catalyzed by creatine kinase. Generally, the initial transient taking over by anaerobic glycolysis of a part of ATP supply from oxidative phosphorylation may be of such a physiological advantage that it gives time for the organism to increase the rate of oxygen delivery to muscle by blood, in order to match the increased oxygen consumption in muscle.

The comparison of Fig. 3b and Fig. 8b demonstrates that anaerobic glycolysis significantly diminishes and shortens the initial alkalization of muscle after an onset of exercise. As it was discussed previously [5], the initial alkalization may lead to an instability of the system, and therefore counter-acting this phenomenon seems to be profitable from the physiological point of view. In fact, it was proposed in the previous article [5] that the preventing the instability of the system is the second, after additional ATP supply, physiological role of anaerobic glycolysis in skeletal muscle. A similar role may be played by the parallel activation of ATP supply and ATP demand [16], which leads to a smaller increase in [ADP] during muscle work and, in consequence, to a smaller consumption of protons by CK during conversion of PCr to Cr. A third mechanism that diminishes the initial alkalization may be proton influx from blood [5].

The results of computer simulations in Mode 3 for transition from rest to intensive exercise (100-fold increase in ATP usage) are presented in Fig. 9. In the simulations concerning the system without anaerobic glycolysis, sudden transition from rest to intensive exercise leads to an instability of the bioenergetic system in muscle [5]. Therefore, the only conclusion

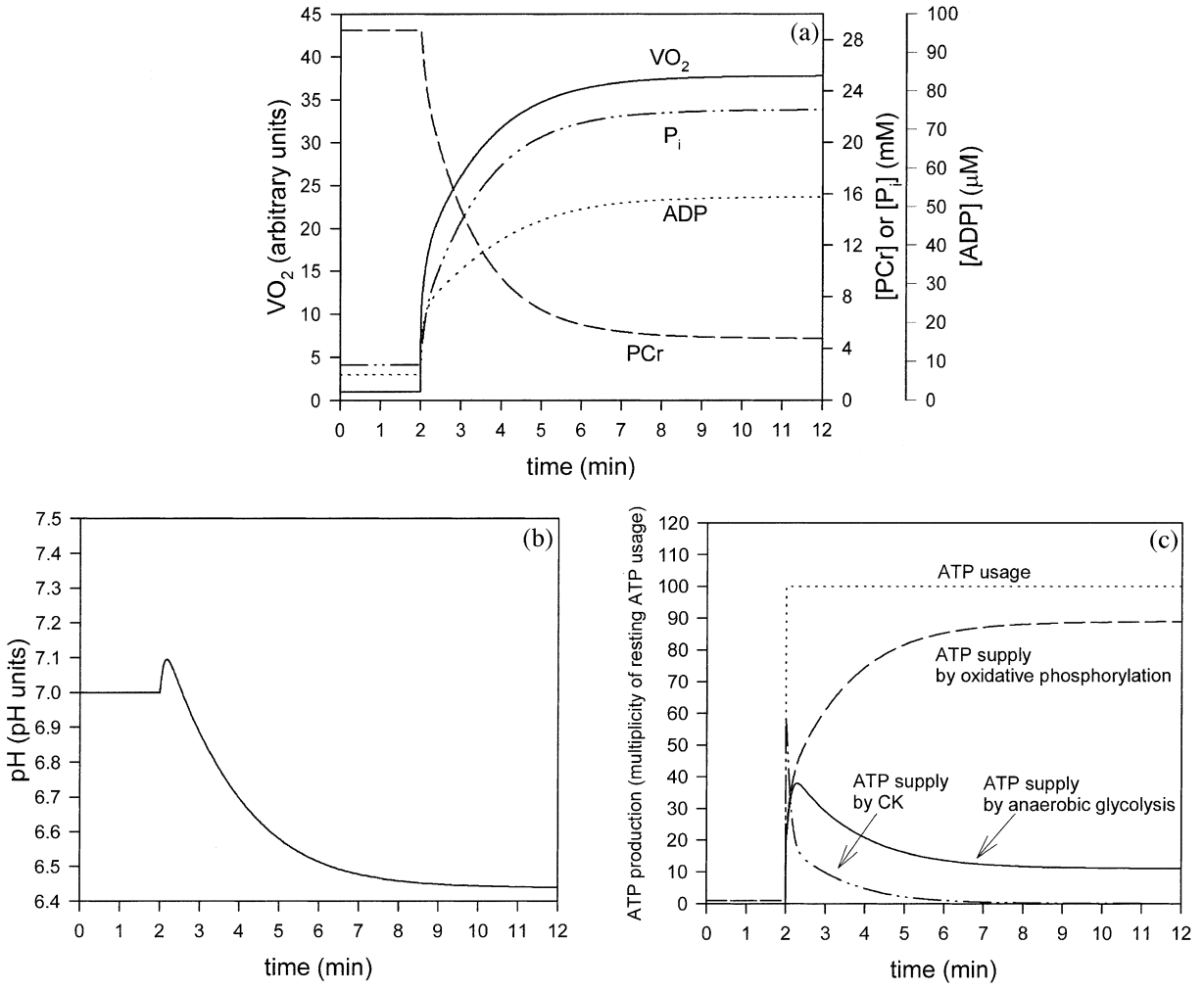

Fig. 9. Simulated behavior of the bioenergetic system in muscle in Mode 3 during transition from rest to intensive exercise. Mode 3: direct activation of glycolysis during muscle contraction, activation of glycolysis by ADP (plus AMP and P_i), inhibition of glycolysis by H^+. After first 2 min of simulation, representing resting steady-state, ATP usage was increased 100 times. In the same moment, glycolysis was directly activated $100^{0.8}$ times and oxidative phosphorylation was activated $100^{0.4}$ times. (a) time courses of V_{O_2} (standardized for 1 in resting state), ADP, PCr and P_i; (b) time course of cytosolic pH; (c) time courses of ATP usage, ATP supply by creatine kinase, ATP supply by anaerobic glycolysis and ATP supply by oxidative phosphorylation (including aerobic glycolysis).

that can be drawn from the comparison of the system without and with anaerobic glycolysis at intensive exercise is that anaerobic glycolysis prevents this instability.

It can be seen in Fig. 9a that the transition time of the respiration rate between rest and work ($t_{1/2}$) is equal to approximately 20–30 s, and that V_{O_2} approaches its maximal value during 3–4 min after an onset of exercise. This theoretical prediction agrees rather well with experimental data, although V_{O_2} tends to reach its maximal value after 2–3 min in experimental studies [4,19]. This small discrepancy may be due to the simplified dependence of the glycolytic flux on H^+ assumed in the present article (Eq. (2))—in reality, anaerobic glycolysis may be inhibited by H^+ earlier than in the computer

simulations presented. In the active steady-state [PCr] decreases five times to 5 mM, while [P_i] rises to over 20 mM. This theoretical result also agrees well with experimental data [8,9].

According to Fig. 9b, the initial alkalization at an onset of intensive exercise reaches maximally 0.1 pH units and lasts 0.5 min. This theoretical prediction also agrees well with the experimental values reported in the literature [8,20–23]. At the same time, a comparison of Fig. 8b and Fig. 9b demonstrates that the time of the lasting of the initial alkalization becomes shorter when the intensity of exercise increases. This theoretical finding is confirmed by experimental data as well [8]. pH drops in the simulation presented in Fig. 9b by over 0.5 pH units. Such a great pH decrease, by 0.5–0.7 pH units [3,8] is observed during exercise of small muscle groups where $\dot{V}O_{2max}$ is very high, but not during exercise involving large muscle groups, e.g. cycling, where $\dot{V}O_{2max}$ is at least twice smaller.

Finally, Fig. 9c demonstrates that creatine kinase is the main ATP-producing process during the first seconds of intensive exercise in Mode 3. After this time, it is quickly replaced as a source of ATP by oxidative phosphorylation (including aerobic glycolysis) and anaerobic glycolysis. The ATP supply by anaerobic glycolysis increases quickly after an onset of exercise (direct activation during muscle contraction plus activation by ADP, AMP, P_i and alkalization), after approximately 20 s reaches a peak, at which almost 40% of ATP is supplied by this process, and then gradually decreases to the steady-state value (inhibition by acidification) equivalent to approximately 12% of the overall ATP supply. The first, very quick phase of the increase in the glycolytic flux is due the direct activation of glycolytic enzymes, while the second phase lasting approximately 20 s is due to the indirect activation of glycolysis, in particular of its enzymes having the greatest control over the glycolytic flux (glycogen phosphatase and phosphofructokinase), by increased metabolite concentrations: [ADP], [AMP] and [P_i] (within the model, the dependence of the glycolytic flux on these metabolite concentrations is represented by the phenomenological dependence on [ADP], as discussed above). As it was already discussed before, this theoretical prediction reflects well (at least semi-quantitatively) the behavior observed in working muscle during experimental studies [3].

3.5. General discussion

The model of oxidative phosphorylation in intact skeletal muscle developed previously by Korzeniewski et al. [5,11] and supplemented in the present article with a simple description of glycolysis based on the comprehensive model of glycogenolysis and glycolysis in skeletal muscle developed recently by Lambeth and Kushmerick [14] contains all the most important components of the bioenergetic system in skeletal muscle: oxidative phosphorylation, anaerobic glycolysis, creatine kinase system (CK/PCr/Cr), ATP usage, proton efflux/influx and proton buffering. Therefore, this model comprises explicitly all the processes contributing to the balance of ATP and H^+ presented in Fig. 1.

In the present work, the emphasis is laid on the regulation of the glycolytic flux and on the influence of anaerobic glycolysis on the rest of the bioenergetic system, especially on oxidative phosphorylation. Computer simulations allowed formulating several interesting conclusions and proposals. First of all, the model showed that it is not possible to explain the kinetic behavior of glycolysis in intact muscle only by the regulation of this process by changes in ADP, AMP, ATP and P_i. It is not possible using the comprehensive model of glycolysis developed by Lambeth and Kushmerick to elevate the glycolytic flux during transition from rest to intensive exercise to the level encountered in intact skeletal muscle. The difference between theoretical predictions and experimental observations is so great that it is unlikely that it is caused by small inaccuracies of the model. It must be emphasized that the discussed discrepancy is even much greater than it can be concluded from the presented computer simulations, because during short-term exercise of maximal intensity, where glycolysis becomes the main source of ATP, the glycolytic flux rises much above the levels considered in the present article.

Of course, any desired increase in the glycolytic flux may be achieved if glycolysis is directly activated to an appropriate extent. Because it is not known which particular glycolytic enzymes are

directly activated and to what extent, it is convenient to use the simplified kinetic description of glycolysis presented in Eq. (1) (Mode 2 of simulations). Nevertheless, computer simulations show that the inclusion to the model of the direct activation of glycolysis still does not give fully satisfactory results. Namely, simulations in Mode 2 predict too great initial alkalization after the onset of exercise and give a sustained (not transient) stimulation of glycolysis during exercise.

However, the model is able to match well (at least semi-quantitatively) the experimental data if the inhibition/activation of glycolysis by acidification/alkalization of the cytosol is taken into account. The version of the model (Mode 3) involving the dependence of the glycolytic flux on H^+ predicts a realistic value of the acidification of muscle during exercise (by approx. 0.2 pH units at moderate exercise and by approx. 0.55 pH units during intensive exercise) and allows generating only a transient activation of anaerobic glycolysis after an onset of exercise.

Therefore, it can be concluded that the regulation of glycolysis in muscle during transition from rest to work is a result of the dynamic balance between the activation of this metabolic pathway by ADP, AMP, P_i and alkalization, direct activation by some cytosolic factor during muscle contraction and the inhibition of glycolysis by acidification.

The mechanisms underlying the direct activation of glycolysis during muscle contraction remain unclear. It is a well documented fact that calcium ions stimulate glycogenolysis by stimulating the transformation of glycogen phosphorylase b to glycogen phosphorylase a [3,24]. However, there exist several evidences against a significant involvement of this mechanism in the direct activation of glycolysis during muscle work [3]. Therefore, Connett and Sahlin have postulated an existence of some other unknown factor/mechanism related to muscle contraction, which directly activates the glycolytic flux [3]. Hochachka [7] proposed that direct activation of particular glycolytic enzymes is necessary to explain the stability of intermediate metabolites of glycolysis during transition between different energetic states. However, Thomas and Fell [25] argued that such a stability can be potentially explained without direct activation, if the sensitivity of the glycolytic flux to ADP (plus AMP and P_i) is great enough. The present study demonstrates [theoretical results not shown] that this does not take place in the case of the model of glycolysis developed by Lambeth and Kushmerick (see above).

The comparison between the simulations performed within Mode 1 (no anaerobic glycolysis) and Mode 3 (glycolysis dependent on ADP and protons, and directly activated during muscle contraction) allows to investigate the influence of the presence of anaerobic glycolysis on the kinetic properties of oxidative phosphorylation and of the whole bioenergetic system in skeletal muscle. First of all, the glycolytic H^+ production is able to prevent the potential instability of the system at high exercise intensities as it was suggested previously [5]. Second, the transiently stimulated ATP supply by anaerobic glycolysis after an onset of exercise significantly slows down the transition of V_{O_2} from rest to work, although the transition times of PCr, ADP and P_i are not significantly affected. Third, the (quasi-)steady-state values of [PCr], [ADP] and [P_i] are changed. Fourth, the cytosol becomes acidified during exercise. Fifth, the initial alkalization becomes diminished and shortened. Sixth, the phase of the initial ATP production by CK is slightly shortened (Fig. 3c vs. Fig. 8c).

The shape of the time course of V_{O_2} and/or [PCr] during rest→work transition or during muscle recovery from exercise is sometimes used to investigate the regulation of oxidative phosphorylation by ADP, Cr, phosphorylation potential and so on [26,27]. An important limitation for this kind of analysis is the possibility of the parallel activation of ATP supply and ATP usage during muscle contraction [12,16,17]. However, the previous article [5] and the present study suggest that, even if the parallel activation is left aside, the shapes of the V_{O_2} and [PCr] transition curves depend on so many factors and processes that it would be extremely difficult to extract any simple quantitative information from them. Namely, these shapes depend both on the properties of the processes that produce ATP, e.g. the displacement of CK from equilibrium, regulation of glycolysis

or relationship between oxidative phosphorylation and [ADP], and on the processes that affect the (changes in the) cytosolic proton concentration: creatine kinase, anaerobic glycolysis, efflux/influx of protons and proton buffering in cytosol. Therefore, steady-state variable values seem to be a better reference point for the analysis of the kinetic properties and behavior of the bioenergetic system in muscle than time courses of these variables in transient states.

Fig. 8 suggests that anaerobic glycolysis is stimulated even at moderate exercise intensities, below the lactate threshold (LT) [28]. Such a possibility has already been proposed by several authors. Brooks [29] suggested that below lactate threshold activation of anaerobic glycolysis is too small to cause noticeable accumulation of lactate in blood. Gollnick et al. [30] as well as Ivy et al. [31] postulated recruitment of some of the glycolytic type II muscle fibers already at low exercise intensities, well below the lactate threshold. However, the kinetic description of the direct activation of glycolysis during muscle contraction used in the present article (n^x) is probably only a rough approximation of the reality and may overestimate the stimulation of glycolysis at moderate work intensities.

The model used in the present work implies that glycolysis is activated by alkalization of the cytosol (Eq. (2); Figs. 8 and 9). This prediction agrees well with the experimental results concerning the effect of induced alkalosis in human skeletal muscle [10].

In the simulations performed in the present work concerning the exercise intensities at which oxidative phosphorylation is still the main source of ATP, the glycolytic ATP production does not exceed approximately 40% of the overall ATP supply after an onset of exercise and approximately 16% of overall ATP production in the active (quasi-)steady-state. However, of course, at extremely high short-time exercises, where ATP usage is essentially greater than the capacity of oxidative phosphorylation for ATP production, anaerobic glycolysis is greatly stimulated and predominates as an ATP-producing process [3]. Such a big activation of glycolysis, which overcomes the inhibition of this process by low pH, may be caused by a very strong direct stimulation of the glycolytic flux by some still unknown factor/mechanism related to muscle contraction. Of course, this would bring about a rapid and large acidification of the cytosol. This fact might by one of the main reasons why extremely intensive exercises are very short—they must be terminated before pH falls below, say, 6.0, in order not to cause a damage of a muscle cell.

It must be emphasized that the computer model of oxidative phosphorylation+glycolysis used in the present study contains several simplifications and approximations. First, a simple linear dependence of the glycolytic flux on [ADP] was extracted from the model developed by Lambeth and Kushmerick. This was justified by the fact that the regulation by ADP, AMP and P_i cannot alone account for the kinetic properties of glycolysis in intact muscle. Such a simple description allowed including in the model the direct activation of glycolysis by some contraction-related factor and the inhibition of this process by protons. Second, a simple linear dependence of the glycolytic flux on $[H^+]$ was assumed (Eq. (2)). However, the real dependence may be more complicated. This may be the reason why the simulated initial transient activation of anaerobic glycolysis lasts a little longer than in reality, and, consequently, causes a longer transition of V_{O_2} to the active steady-state than that observed in experimental studies [4,19]. Third, a simplified kinetic description of the direct activation of glycolysis (n^x) was used. Fourth, the slow component in the oxygen uptake kinetics, observed in the intact muscle above the lactate threshold [32], is not taken into account within the model. Fifth, it is assumed that oxidative phosphorylation is directly activated by some external cytosolic factor (e.g. calcium ions) instantly after an onset of exercise. This is the reason of the very quick initial increase in the respiration rate. However, this is most probably only a rough approximation of the real situation, because calcium needs some time to enter mitochondria and to activate mitochondrial enzymes.

For all these reasons, all the predictions produced by the discussed model should be treated as semi-quantitative ones. The present study is intended to deal with the kind of the mechanisms regulating glycolysis during muscle contraction, and of the influence of anaerobic glycolysis on

the kinetic properties of oxidative phosphorylation, rather than with the precise quantitative predictions. However, the general good agreement between computer simulations and experimental data seems to suggest that the model describes well different aspects of the behavior of the energetic system of skeletal muscle during transition from resting state to exercise.

Generally, in the present work, first version of a computer model was developed which takes into account all the most important elements of the bioenergetic system in muscle: oxidative phosphorylation, creatine kinase, anaerobic glycolysis, efflux/influx of protons and buffering of protons in cytosol. This model can be improved and extended in many aspects and directions. First, a complete, detailed kinetic description of the tricarboxylate acid cycle (TCA), glycolysis and fatty acid β-oxidation can be incorporated into this model. Second, the model can be extended in order to simulate the behavior of the system during extremely intensive short-term exercise, where anaerobic glycolysis is the predominant source of ATP. Third, the factors causing the slow component of oxygen uptake kinetics and muscle fatigue can be included. It is planned to develop the model in the above directions in the future.

4. Conclusions

In the present article, the comprehensive model of glycolysis in skeletal muscle developed by Lambeth and Kushmerick, describing the glycolytic flux as a function of [ADP], [AMP], [ATP] and [P_i], was incorporated to the model of oxidative phosphorylation in intact skeletal muscle developed by Korzeniewski et al. Computer simulations using the joined model strongly suggested that the regulation of glycolysis by adenine nucleotides and inorganic phosphate cannot account for the great increase in the glycolytic flux encountered in muscle during transition from rest to intensive exercise, and therefore a direct activation of glycolysis by some cytosolic factor during muscle contraction is necessary.

Therefore, a simple kinetic description of the dependence of glycolysis on ADP (plus AMP, ATP and P_i) was extracted form the above joined model and supplemented with the postulated direct activation of glycolysis during muscle contraction and with the inhibition of this process by H^+. Such a description was incorporated to the model of oxidative phosphorylation in intact muscle developed previously and thus a computer dynamic model of the entire bioenergetic system in skeletal muscle was developed. The extended model was used to a number of theoretical studies concerning the regulation of glycolysis during muscle contraction and the influence of this metabolic pathway on oxidative phosphorylation.

The computer simulations performed suggest that the activation of glycolysis by ADP, AMP and P_i during transition from rest to work plus the direct activation of glycolysis during muscle contraction alone cannot account for the behavior of the system in intact muscle. In order to explain the transient activation of glycolysis after an onset of exercise and to predict the correct extent and duration of the initial alkalization the inhibition of the glycolytic flux by H^+ ions must be taken into account. Therefore, the regulation of glycolysis during transition from rest to exercise can be characterized as a competition between the direct activation of this process by some contraction-related factor, the activation of glycolysis by ADP, AMP and P_i (and initial alkalization) and the inhibition of glycolysis by acidification of cytosol. Of course, the physical nature of the contraction-related factor that directly activates glycolysis remains unknown and the extent of this activation was adjusted within the model in order to obtain a good agreement of computer simulations with experimental results. The present paper was mainly intended to show that something is lacking in our understanding of the functioning of the bioenergetic system in muscle. Therefore, the problem of the regulation of glycolysis in skeletal muscle certainly needs further experimental and theoretical studies.

Theoretical investigations also show that anaerobic glycolysis prevents the instability of the system that could appear at intensive exercise, slows down the transition of $\dot{V}O_2$ from resting value to working (quasi-)steady-state value and diminishes the extent and the time of duration of the initial alkalization of muscle after an onset of exercise. Generally, a good,

at least semi-quantitative agreement between the theoretical predictions and experimental date takes place.

Summing up, the discussed computer model of the energetic system in skeletal muscle seems to be a useful tool for theoretical studies concerning kinetic properties and regulation of particular metabolic pathways entering into the composition of this system. The model can be further extended in the future, and its theoretical predictions can be tested in the experimental way.

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