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ORIGINAL PAPER

Computer-aided analysis of biochemical mechanisms that increase metabolite and proton stability in the heart during severe hypoxia and generate post-ischemic PCr overshoot

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Abstract During severe hypoxia in the heart, impaired supply of ATP by oxidative phosphorylation could lead to a great drop in ATP turnover and heart work. Anaerobic glycolysis enables unchanged ATP turnover to be maintained, but leads to huge changes in metabolite (PCr, ATP, ADP, P_i) concentrations and to cytosol acidification. A computer model of heart energetics developed previously is used to analyze semi-quantitatively the effect of different processes/mechanisms that can partly counteract these effects. Down-regulation of ATP usage compromises cardiac output, but reduces changes in cytosolic pH and metabolite concentrations. AMP decomposition delays cytosol acidification but reduces metabolite homeostasis (concentration stability). An increase in the parallel activation of oxidative phosphorylation (OXPHOS) (a hypothetical mechanism involving direct activation of all OXPHOS complexes by a cytosolic factor, postulated to take place also during work increase) reduces cytosol acidification and elevates metabolite homeostasis. All these mechanisms can generate the post-ischemic PCr overshoot.

Keywords Heart ischemia · PCr overshoot · ATP usage down-regulation · AMP decomposition · Parallel activation · Ischemic preconditioning

Abbreviations

OP	Oxidative phosphorylation
AG	Anaerobic glycolysis
down-reg.	Down-regulation of ATP usage
AMP deco.	AMP decomposition
parallel act.	Parallel activation of oxidative phosphorylation
PCr	Phosphocreatine
ΔG_P	Phosphorylation potential
Δp	Protonmotive force
IPC	Ischemic preconditioning
RPP	Rate–pressure product
LVDP	Left ventricular developed pressure

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Introduction

Oxidative phosphorylation in mitochondria is essentially the only source of ATP in the heart under normal (in particular, normoxic) physiological conditions. In the steady-state, fluxes (ATP turnover, oxygen consumption, cardiac output), metabolite (ATP, ADP, P_i, PCr, Cr, NADH, etc.) concentrations, and cytosolic pH are maintained at a constant level (metabolite concentrations and pH do not change significantly even during large-scale work transitions [1–4]). However, during ischemia leading to severe hypoxia significant changes in these variables take place:

1 heart work (expressed as cardiac output, rate-pressure product (RPP), or left ventricular developed pressure (LVDP)), ATP turnover, and oxygen consumption can drop substantially [5–11];
2 pH decreases by up to 1 pH unit [5–13]; and
3 PCr, ATP, and phosphorylation potential ΔG_P (proportional to log([ATP]/[ADP][P_i]) decrease, whereas ADP and P_i increase substantially [5–13].

Most of these changes are harmful for cardiac myocytes and the whole organism. A decrease in cardiac output can

lead to hypoxia in other tissues/organs, which is particularly dangerous for the brain. Huge cytosol acidification can seriously damage heart cells. A decrease in the phosphorylation potential ΔG_P can disturb heart functioning and lead not only to a further limitation of cardiac output, but also to a damage of heart cells by stopping the basic reactions keeping them alive and by dissipating ion gradients across the cellular membrane. Therefore, the heart has to optimize its response to hypoxia in order to minimize the harm brought about by it.

Several processes related to heart bioenergetics are recruited in the heart during severe hypoxia. First, substantial activation of ATP supply by anaerobic glycolysis occurs. This helps to keep ATP turnover flux and cardiac performance unaffected (at least for some time) but leads to a substantial decrease in the phosphorylation potential ΔG_P (decrease in PCr and ATP, increase in ADP, AMP, and P_i) and substantial cytosol acidification. Previous in-silico studies [14] have demonstrated that the AMP deamination + adenylate kinase system delays cytosol acidification in skeletal muscle during intensive exercise but leads to a decrease in the ATP/ADP*P_i ratio and thus ΔG_P. (AMP deamination to IMP is the main way of AMP decomposition in skeletal muscle; in heart AMP is mostly decomposed by dephosphorylation to adenosine, see below). Finally, it is possible that the (still hypothetical) parallel activation of different oxidative phosphorylation complexes proposed previously is enhanced during severe hypoxia and thus helps to maintain heart homeostasis (constant cardiac output, metabolic fluxes, metabolite concentrations, pH). The parallel activation mechanism, consisting in direct activation of all oxidative phosphorylation complexes together with ATP usage and substrate dehydrogenation (NADH supply) block by a cytosolic factor related to muscle contraction, has been postulated to be the main mechanism responsible for the adjusting the supply of ATP to satisfy ATP demand during work transitions in heart and skeletal muscle ([15–18]; reviewed in Ref. [19]).

Frequently, after a period of ischemia the PCr concentration rises above its normoxic level and remains elevated for many minutes or even hours [5–8, 10, 12, 13]. This is the so-called "post-ischemic PCr overshoot". The biochemical background of this phenomenon is still unexplained.

Ischemic preconditioning (IPC) caused by prior ischemia periods improves the stability of metabolite (PCr, ATP, P_i) concentrations and of pH during subsequent ischemia periods, and accelerates system (function) recovery after ischemia [6, 7, 10, 11]. IPC can also lead to enlargement of the PCr post-ischemic overshoot. The mechanisms responsible for this conditioning are still not well understood.

The purpose of this theoretical study was to analyze semi-quantitatively the effect of significantly lowered oxygen concentration on the oxidative phosphorylation system and of the enumerated processes/mechanisms: anaerobic glycolysis, ATP demand down-regulation, AMP decomposition, and parallel activation enhancement on different system variables: the fluxes (ATP turnover proportional to cardiac output, oxygen consumption), metabolite (ATP, ADP, P_i, PCr) concentrations, and cytosolic pH in response to severe hypoxia in heart. (It was not the objective of the study to analyze in detail the kinetics of anaerobic glycolysis, ATP demand down-regulation, and AMP decomposition). The effect of particular processes/mechanisms is usually "contradictory": they improve some system variable values, but make worse other variable values. It is therefore hypothesized that the heart chooses a strategy in which the combined harmful effect of a decrease in cardiac output, metabolite (ATP, ADP, P_i, PCr) concentrations, and pH is smallest.

Of course, the general, qualitative role and effect of the processes considered in this study are broadly known. However, to my knowledge, their relative effect on the fluxes and on metabolite and proton concentrations during hypoxia has not yet been estimated. Also the possible mechanisms underlying the post-ischemic PCr overshoot have not been analyzed previously. Therefore, this study is significant progress in understanding the functioning and regulation of the heart bioenergetic system during hypoxia.

Theoretical procedures

Computer model

The well-tested computer model of the bioenergetic system of the heart cell developed previously [16, 17] was used for theoretical studies. It was slightly modified in order to involve anaerobic glycolysis and AMP decomposition, analogously to the model version for skeletal muscle [14, 20]. This model comprises the following main processes: oxidative phosphorylation, glycolytic ATP supply, creatine kinase (CK), ATP usage, and proton efflux.

The kinetic description of oxidative phosphorylation contained in the model comprises explicitly the following complexes: complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, phosphate carrier, and proton leak. The kinetic descriptions of creatine kinase and ATP usage are also included, as described previously [16].

The rate of glycolysis ($\mu M\ min^{-1}$) was described as linearly dependent on cytosolic ADP concentration [20], by use of the following equation (inhibition by protons was not assumed, because it resulted in glycolysis stimulation that was too small):

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

where $k_{GLYC} = 32.19\ min^{-1}$, is the rate constant and ADP_{te} is the total (magnesium-bound and magnesium-free) cytosolic ADP concentration.

The rate of proton efflux from cytosol to blood ($\mu M\ min^{-1}$) was described, as in Ref. [21], by use of the equation:

$$v_{EFFL} = k_{EFFL} \times (pH_0 - pH) \quad (2)$$

where $k_{EFFL} = 10\ mM\ min^{-1}$, is the rate constant and $pH_0 = 7.0$.

The rate of AMP decomposition by dephosphorylation to adenosine and deamination to IMP during hypoxia was described, in the same way as AMP decomposition by deamination in skeletal muscle in Ref. [14], by use of the simple equation:

$$v_{DEAM} = c \quad (3)$$

where $c = 0.24\ mM\ min^{-1}$, was assumed in order to reduce the total adenine nucleotide pool by approximately 50% during 15 min of hypoxia. This is rather a high value (in order to show the qualitative effect as clearly as possible), but still within the range encountered in experimental studies (compare Table 2). Another simulation was performed with the adenine nucleotide pool reduced by a factor of 2 ($c = 0.12\ mM\ min^{-1}$); this affected the results quantitatively, but not qualitatively (see "Theoretical results"). During recovery after hypoxia the resynthesis of AMP was described by assuming $c = -0.12\ mM\ min^{-1}$.

It was assumed that down-regulation of ATP usage leads to an instant 50% decrease of the rate constant of ATP usage (k_{UT}) at the beginning of the hypoxic period. Again, this value is rather high (in order to show the qualitative effect as clearly as possible), but still within the range of experimental values (as can be seen in Table 2). Smaller values cause smaller deviations of the behavior of the system from that observed without any down-regulation (see "Theoretical results"). During recovery the original value of this constant was slowly restored, by use of the equation:

$$u = 1 - (1 - 0.5) \times e^{-t/\tau u} \quad (4)$$

where u is the multiplicity of the initial (normoxic) k_{UT} ($u = 1$ in normoxia, $u = 0.5$ in hypoxia), $\tau u = 30$ min is the characteristic ATP usage recovery time, and t is the time from the onset of recovery.

The enhancement of parallel activation of oxidative phosphorylation during hypoxia consisted in an m-fold ($m = 5$) increase in the rate constants of all oxidative phosphorylation complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, phosphate carrier) and substrate dehydrogenation during hypoxia. During recovery after hypoxia the rate constant values returned exponentially to the initial (normoxic) values:

$$m = 1 + 4 \times e^{-t/\tau} \quad (5)$$

where m is the multiplicity of the initial (normoxic) rate constant, $\tau = 5$ min is the characteristic decay time of the parallel activation (see also Ref. [22]), and t is the time from the onset of recovery. The value of τ was taken from simulations for skeletal muscle [22]; however, it is likely that in the heart this time can be much longer, at least in some cases, because the duration of the post-ischemic PCr overshoot can be tens of minutes (see below). The higher the value of m, the greater the effect of parallel activation, i.e. the greater the deviation of the behavior of the system from that observed without any enhancement of parallel activation (see "Theoretical results").

The complete model description of the heart bioenergetic system including anaerobic glycolysis is located on the web site: http://awe.mol.uj.edu.pl/~benio/.

Computer simulations

The dependence of fluxes and metabolite concentrations on oxygen concentration (Fig. 1) was simulated by setting different oxygen concentrations and recording appropriate steady-state variable values (the system approached a new steady state after a sufficiently long time). The simulations were performed in Mode 1 with varying oxygen concentration (see below).

Time courses of ATP fluxes and metabolite concentrations during normoxia, severe hypoxia, and recovery after hypoxia (Figs. 2, 3, 4, 5, 6) were simulated by recording the normoxic steady state (saturated $[O_2] = 240\ \mu M$) for the first 2 min, reducing $[O_2]$ to 0.1 μM for the next 15 min (severe hypoxia), and then restoring the saturated oxygen concentration for the rest of the simulation (subsequent 28 min, recovery after hypoxia).

Five different modes of simulation were used. In Mode 1 (Fig. 2) it was assumed there was no anaerobic glycolysis and, therefore, ATP is delivered solely by oxidative phosphorylation and, in transient states, by creatine kinase (OP Mode). In Mode 2 (Fig. 3) anaerobic glycolysis was present (OP + AG Mode). In Mode 3 (Fig. 4) down-regulation of ATP usage was introduced to the system containing oxidative phosphorylation and anaerobic glycolysis (OP + AG + down-reg. Mode). In Mode 4 (Fig. 5) AMP decomposition was introduced to the system containing oxidative phosphorylation and anaerobic glycolysis (OP + AG + AMP deco. Mode). Finally, in Mode 5 (Fig. 6) the hypothetical parallel activation of different oxidative phosphorylation complexes was introduced to the system containing oxidative phosphorylation and anaerobic glycolysis (OP + AG + parallel act. Mode).

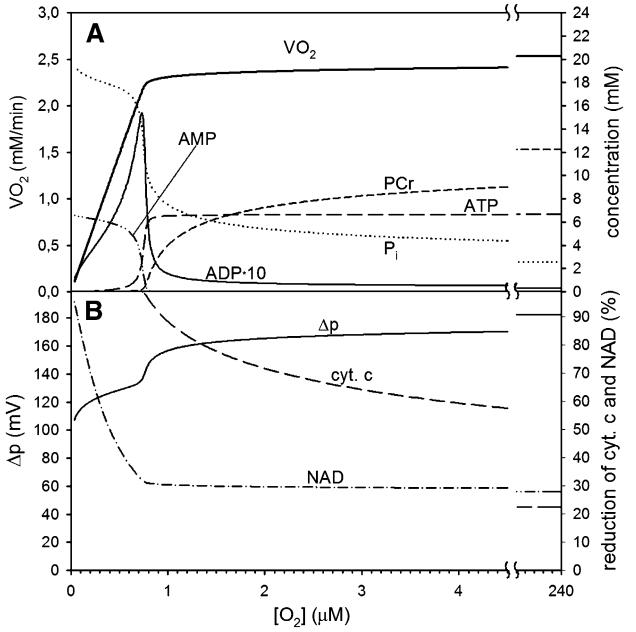

Fig. 1 Simulated dependence of the respiration rate (VO_2) and metabolite concentrations on oxygen concentration ($[O_2]$) in the heart. **a** Dependence of VO_2, ATP, ADP, AMP, P_i, and PCr; **b** dependence of the protonmotive force (Δp), cytochrome *c* reduction level, and NAD reduction level

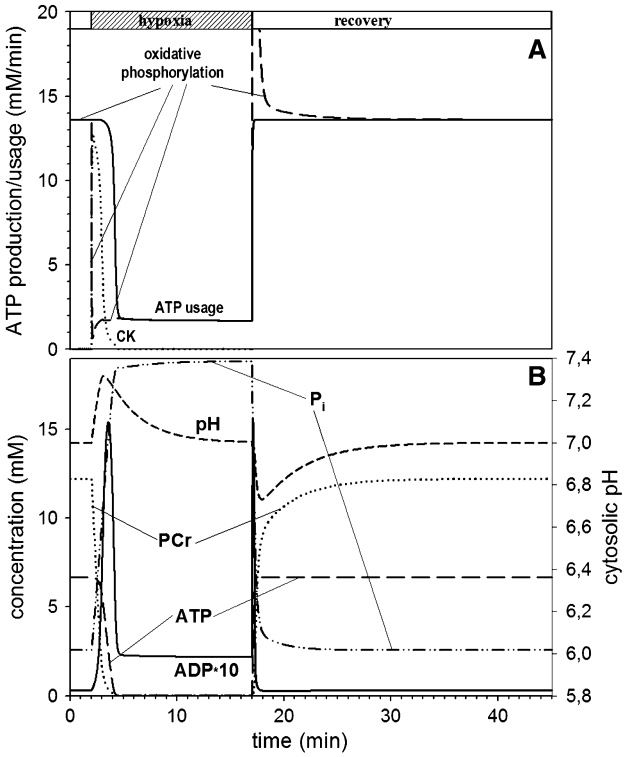

Fig. 2 Simulated time courses of ATP fluxes and metabolite concentrations during normoxia, hypoxia, and recovery after hypoxia in Mode 1 (OP). 0–2 min, normoxia; 2–17 min, hypoxia; 17–45 min, recovery after hypoxia. **a** Time courses of ATP usage and ATP production by oxidative phosphorylation and creatine kinase (CK); **b** time courses of ATP, ADP, PCr, P_i, and pH

Another simulation was also performed. This represented combined Modes 3 and 4 (Mode 3 + 4 = OP + AG + down-reg. + AMP deco. Mode). This means that both ATP usage down-regulation and AMP decomposition were included in this simulation. The quantitative extent of these processes was identical with those in the "original" Modes 3 and 4.

Theoretical results

The rate of respiration (and, consequently, oxidative ATP production) is essentially independent of oxygen concentration in a very broad range. This is demonstrated in Fig. 1a. Oxygen consumption starts to decrease significantly when $[O_2]$ falls below 1 μM (the saturating oxygen concentration is 240 μM). However, metabolite concentrations begin to change at much higher oxygen concentrations (Fig. 1a, b). In particular, PCr and Δp decrease with decreasing $[O_2]$, whereas ADP, P_i, AMP, and the reduction level of cytochrome c and NAD increase. At very low oxygen concentrations ADP decreases, because it is converted to AMP by the reaction catalyzed by adenylate kinase (under those conditions almost all adenine nucleotide molecules are in the form of AMP, because of the equilibrium constant of this enzyme). Generally, similar behavior of the oxidative phosphorylation system was observed in experimental studies [23]. It has been proposed [24] that the great insensitivity of VO_2 to $[O_2]$ is caused by compensation of the fall in $[O_2]$, which inhibits the reaction catalyzed by cytochrome oxidase, by an increase in cytochrome c reduction and a decrease in Δp that activate cytochrome oxidase. The reduced form of cytochrome *c* is a substrate of this enzyme, whereas Δp (related to the proton gradient across the inner mitochondrial membrane) is its product. Therefore, a decrease in the concentration of one substrate (O_2) is counter-balanced (by approx. 90% [24]) by an increase in another substrate concentration (cyt.c^{2+}) and by a decrease in a product concentration (Δp). As a result the half-saturation constant $K_{0.5}$ of oxidative phosphorylation for oxygen is below 1 μM in intact cells and even below 0.1 μM in isolated mitochondria [25].

In computer simulations representing the normoxia–hypoxia–recovery transition (Figs. 2, 3, 4, 5, 6), the oxygen concentration of 0.1 μM was chosen, to cause very severe hypoxia in which the ATP production by oxidative phosphorylation is dramatically reduced. $[O_2]$ was instantly reduced from 240 to 0.1 μM at the onset of hypoxia, and instantly restored to 240 μM at the beginning of post-hypoxic recovery.

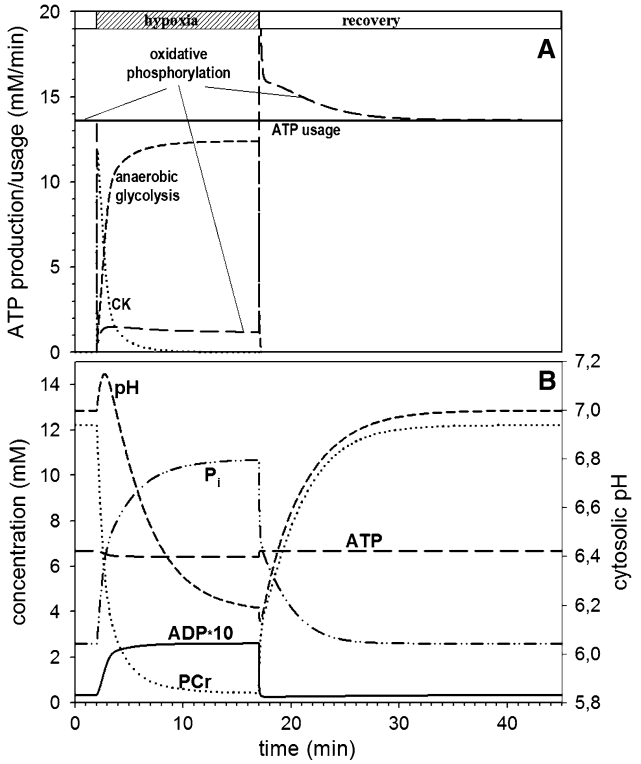

Fig. 3 Simulated time courses of ATP fluxes and metabolite concentrations during normoxia, hypoxia, and recovery after hypoxia in Mode 2 (OP + AG). 0–2 min, normoxia; 2–17 min, hypoxia; 17–45 min, recovery after hypoxia. **a** Time courses of ATP usage and ATP production by oxidative phosphorylation, creatine kinase (CK), and anaerobic glycolysis; **b** time courses of ATP, ADP, PCr, P_i, and pH

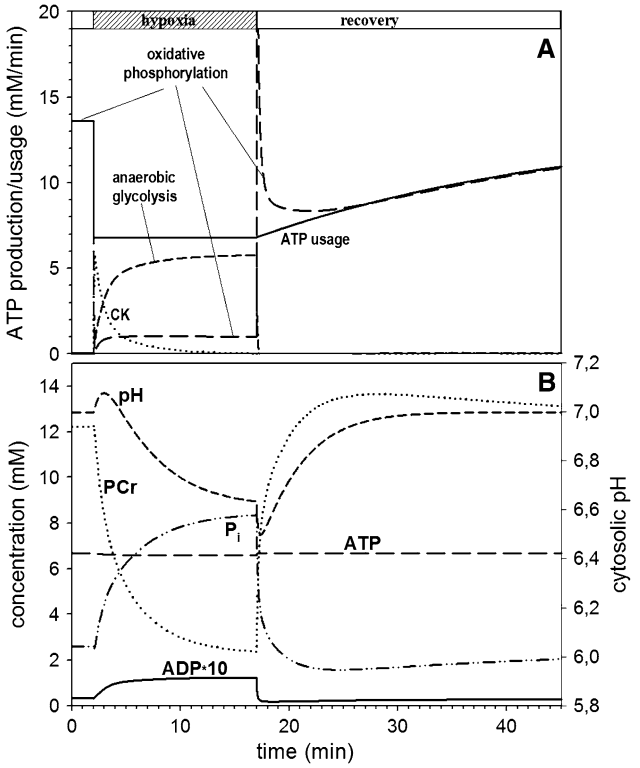

Fig. 4 Simulated time courses of ATP fluxes and metabolite concentrations during normoxia, hypoxia, and recovery after hypoxia in Mode 3 (OP + AG + down-reg.). 0–2 min, normoxia; 2–17 min, hypoxia; 17–45 min, recovery after hypoxia. **a** time courses of ATP usage and ATP production by oxidative phosphorylation, creatine kinase (CK), and anaerobic glycolysis; **b** time courses of ATP, ADP, PCr, P_i, and pH

First, computer simulations were carried out for a "reference" situation in which ATP is produced only by oxidative phosphorylation (and, in transient states, by CK) (Mode1: OP). Of course, this is a theoretical situation that does not take place in normal hearts. In the absence of ATP supply by anaerobic glycolysis severe hypoxia leads to a complete collapse of the bioenergetic system in the heart. This is demonstrated in Fig. 2 and Table 1. During hypoxia the ATP turnover flux (= ATP usage = ATP supply in a steady-state) decreases to approximately 15% of its normoxic value (this is equivalent to the capacity of oxidative phosphorylation at lowered O_2). ATP and PCr are almost completely exhausted, whereas P_i almost reaches its maximum level. On the other hand, pH initially rises, because of proton consumption by the CK-catalyzed reaction, but afterwards returns to the normoxic value (7.0). During recovery after hypoxia all variables return to their initial (normoxic) values. The highly activated oxidative phosphorylation after the onset of recovery (high [O_2] plus elevated [ADP]) supply ATP for both heart work and PCr resynthesis. pH transiently decreases because of proton release by the reverse CK reaction.

The presence and activation of anaerobic glycolysis during hypoxia (Mode 2: OP + AG) substantially changes the behavior of the system. This can be seen in Fig. 3 and Table 1. Now the initial ATP turnover is maintained during hypoxia because most of the ATP is supplied by anaerobic glycolysis. ATP remains almost unchanged. A significant decrease in PCr and increase in P_i occur, but not to almost zero and the maximum value, respectively, as in Mode 1. A moderate increase in ADP activates anaerobic glycolysis (compare Eq. 1). However, in this mode huge acidification of the cytosol takes place during hypoxia: pH decreases by 0.81 pH units, from 7.0 in normoxia to 6.19 after 15 min of hypoxia. The pH decrease slows down with time because of activation by the low pH of proton efflux from the heart cells to the blood (compare Eq. 2).

The down-regulation of ATP usage by 50% during hypoxia in the presence of anaerobic glycolysis (Mode 3: OP + AG + down-reg.) also leads to a decrease in ATP turnover by 50%. At the same time, the stability of PCr, ADP, and P_i is improved. This can be seen in Fig. 4 and Table 1. However, what is more important, down-regulation of ATP usage leads to a significant decrease in cytosol acidification: in this case pH decreases by 0.36 pH units only, from 7.0 to 6.64. During recovery after hypoxia,

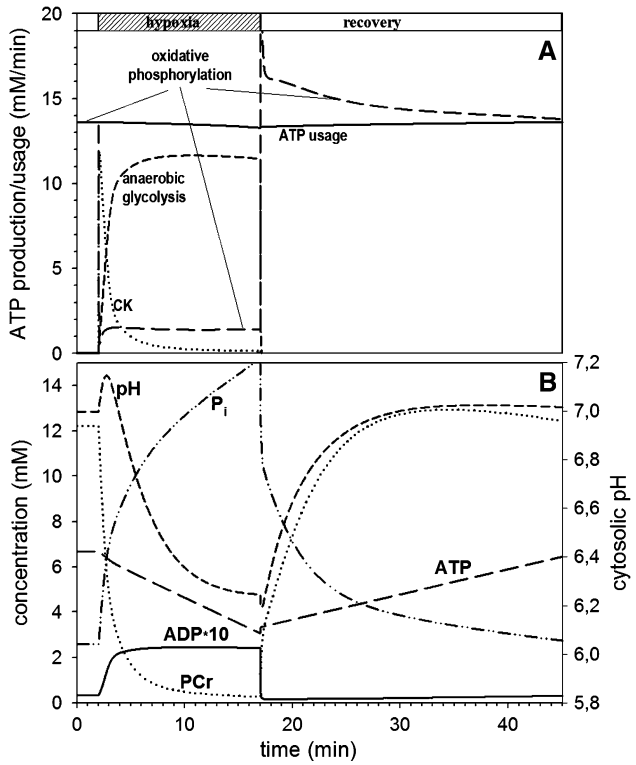

Fig. 5 Simulated time courses of ATP fluxes and metabolite concentrations during normoxia, hypoxia, and recovery after hypoxia in Mode 4 (OP + AG + AMP deco.). 0–2 min, normoxia; 2–17 min, hypoxia; 17–45 min, recovery after hypoxia. **a** Time courses of ATP usage and ATP production by oxidative phosphorylation, creatine kinase (CK), and anaerobic glycolysis; **b** time courses of ATP, ADP, PCr, P_i, and pH

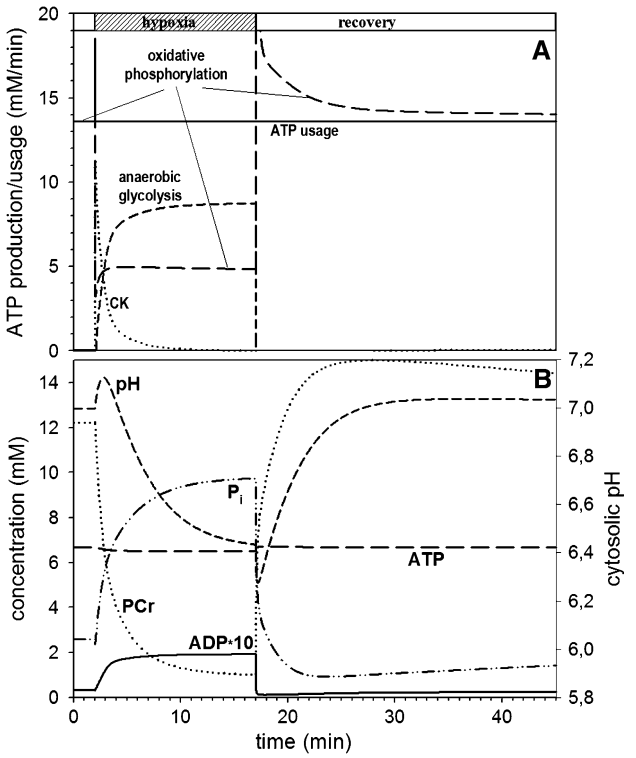

Fig. 6 Simulated time courses of ATP fluxes and metabolite concentrations during normoxia, hypoxia, and recovery after hypoxia in Mode 5 (OP + AG + parallel act.). 0–2 min, normoxia; 2–17 min, hypoxia; 17–45 min, recovery after hypoxia. **a** Time courses of ATP usage and ATP production by oxidative phosphorylation, creatine kinase (CK), and anaerobic glycolysis; **b** time courses of ATP, ADP, PCr, P_i, and pH

fluxes and metabolite concentrations return to the initial (normoxic) values. The highly activated oxidative phosphorylation at the onset of recovery (high [O_2] plus elevated [ADP]) produces much ATP that is partly used for a quick PCr resynthesis. During post-hypoxic recovery the slow return of the activity of ATP usage to its initial value results in a quite significant PCr overshoot. The duration of the overshoot depends on the rate of return of ATP usage to its initial value: the slower the return, the longer the overshoot (not shown).

When down-regulation of ATP usage by 25% was assumed in a simulation, the deviation of the behavior of the system from that observed in Mode 2 (OP + AG) (Fig. 3) was smaller than in Fig. 4, but the difference was only quantitative. In particular, changes in [PCr], [P_i], [ADP], and pH were higher, and the PCr overshoot was smaller than in Fig. 4 (not shown).

AMP decomposition in the presence of anaerobic glycolysis (Mode 4: OP + AG + AMP deco.) has no effect on the ATP turnover flux. This process (when coupled with adenylate kinase) supplies some ATP (explained in detail elsewhere [14]) and therefore reduces ATP delivery by anaerobic glycolysis at a fixed ATP demand. AMP decomposition reduces metabolite (ATP, PCr, P_i) stability, because it reduces the total adenine nucleotide (ATP + ADP + AMP) pool, which, under normal conditions, is dominated by ATP (because of the adenylate kinase equilibrium). This is shown in Fig. 5 and Table 1. However, it slightly delays cytosol acidification (by reducing glycolytic flux, as mentioned above) which was postulated to be its main role [14]: here pH falls by 0.75 pH units, from 7.0 to 6.25 (Fig. 5; Table 1). When half this rate of AMP decomposition was assumed, the behavior of the system changed substantially, being intermediate between Fig. 3 (Mode 2) and Fig. 5 (for instance, changes in P_i were smaller than in Fig. 5, but greater than in Fig. 3) (not shown). During posthypoxic recovery the slow resynthesis of AMP and, consequently, of the entire adenylate nucleotide pool (mostly ATP) results in a small PCr overshoot. Its duration depends on the rate of AMP (and thus adenylate nucleotide pool) resynthesis: the slower the resynthesis, the longer the PCr overshoot (not shown).

Hypothetical enhancement of the parallel activation of oxidative phosphorylation complexes (Mode 5: OP +

Table 1 Simulated initial (normoxic) and end-of-hypoxia (15 min) values of pH, PCr, ATP, P_i, and ADP in the presence of different processes activated during severe hypoxia in the heart (Modes 1–5)

Conditions	pH	PCr (mM)	ATP (mM)	P_i (mM)	ADP (μM)
Initial state (normoxia) end of hypoxia	7.00	12.22	6.69	2.60	31.7
OP	7.00	0.01	0.02	18.85	220
OP + AG	6.19	0.43	6.41	10.68	260
OP + AG + down-reg.	6.64	2.37	6.57	8.35	123
OP + AG + AMP deco.	6.25	0.25	3.06	15.17	241
OP + AG + parallel act.	6.43	1.00	6.49	9.74	192

OP, oxidative phosphorylation; AG, anaerobic glycolysis; down reg., down-regulation of ATP usage; AMP deco., AMP decomposition; parallel act., (increase in) parallel activation of oxidative phosphorylation

AG + parallel act.) does not affect the ATP turnover flux and leads to elevated stability of metabolite concentrations and significantly smaller cytosol acidification. This is presented in Fig. 6 and Table 1. In this case, PCr decreases and ADP and P_i increase significantly less during hypoxia than in Mode 2 (without parallel activation enhancement). At the same time, pH decreases by only 0.57 pH units, from 7.0 to 6.43. During recovery after hypoxia, when enhancement of parallel activation decays slowly (compare Eq. 4), a huge PCr overshoot occurs.

The effect of parallel activation is proportional to the value of m, the measure of the enhancement of parallel activation. The higher the value of m, the better the stability of pH and metabolite concentrations (not shown). The magnitude of the post-ischemic PCr overshoot also depends on m: the greater the enhancement, the bigger the overshoot (not shown). Its duration depends on the characteristic decay time of the enhancement τ: the longer the time, the longer the overshoot (not shown). Generally, every required duration of the overshoot can be achieved by adjustment of an appropriate characteristic decay time. A detailed analysis of the dependence of the magnitude and duration of the PCr recovery overshoot in skeletal muscle on these different values is presented in Ref. [29].

The simulation combining Modes 3 and 4 gives, as can be expected, theoretical results that are in some respects intermediate and in some respects additive in relation to the results of simulations in Mode 3 and Mode 4. Intermediate was, e.g., the extent and time course of the changes in [PCr] and [P_i]. Additive was the delay in cytosol acidification (pH dropped only by 0.29 pH units, to 6.71, at the end of hypoxia) and the extent of the post-ischemic PCr overshoot (it equaled 18%). This is demonstrated in Fig. 7.

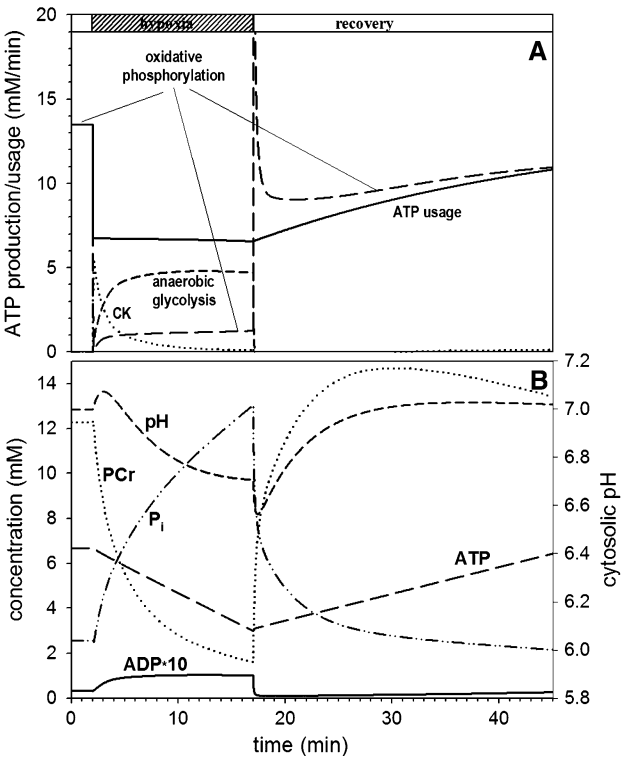

Fig. 7 Simulated time courses of ATP fluxes and metabolite concentrations during normoxia, hypoxia, and recovery after hypoxia in combined Mode 3 + 4 (OP + AG + down-reg. + AMP deco.). 0–2 min, normoxia; 2–17 min, hypoxia; 17–45 min, recovery after hypoxia. **a** Time courses of ATP usage and ATP production by oxidative phosphorylation, creatine kinase (CK), and anaerobic glycolysis; **b** time courses of ATP, ADP, PCr, P_i, and pH

Discussion

In this study the potential effects of different processes/mechanisms recruited in the heart during severe hypoxia (anaerobic glycolysis, ATP usage down-regulation, AMP decomposition, parallel activation enhancement) have been studied semi-quantitatively using the computer model of the bioenergetic system of the heart developed previously, after slight modification. The main question to be answered is what is the real situation prevailing in the heart during severe hypoxia: the relative contribution and the effect of the analyzed processes/mechanisms on the kinetic behavior of the heart bioenergetic system, and their potential to

account for the post-ischemic PCr overshoot observed experimentally.

Generally, good, semi-quantitative agreement between the computer simulations presented in Figs. 3, 4, 5 and 6 and experimental data [5–8, 10–13] can be observed. The values of variables associated with the normoxia–hypoxia–normoxia transition, taken from selected references, are compared in Table 2. Computer simulations carried out in Modes 2–5 involve recruitment of anaerobic glycolysis; Mode 1, which does not involve this process is completely unrealistic. Experimental data for hypoxia lasting approximately 15 min (as in simulations) have been chosen. The variables studied were changes in metabolite (PCr, ATP, P_i) concentrations during transition to hypoxia, changes in pH, time in which [PCr] falls to 50% of its initial value (related to the rate of PCr depletion), decrease in heart work (defined as a decrease in RPP, LVDP, or VO_2, depending on the experiment) versus ATP usage down-regulation (within the model) and the magnitude of the PCr post-ischemic overshoot. The simulated changes in [PCr] and [P_i] are well within the range encountered in experimental studies. If no AMP decomposition is assumed (Modes 2, 3, 5) then changes in [ATP] are tiny, much smaller than those found experimentally. This suggests that substantial AMP decomposition is always present during severe hypoxia in the heart. The rate of AMP decomposition assumed in Mode 4 gives a drop in ATP that is within the range of experimental values, perhaps closer to the higher extreme of this range. The simulated range of pH changes (by approx. 0.4–0.8 pH units, 0.29 in combined Mode 3 + 4) covers approximately the typical range observed in experiments. The initial rate of PCr decomposition, characterized by the time of fall of [PCr] to 50% of the initial value, reproduces the experimental findings well. The decrease in the ATP usage intensity proportional to cardiac output assumed in Mode 3 (50%) is within the values seen in experimental results (perhaps closer to the higher extreme), whereas no down-regulation of ATP usage, as assumed in other modes, seems to be unrealistic (although in some cases this decrease can be as small as 10%, as in Ref. [7]). Finally, the typical value of the PCr post-ischemic overshoot seen in experiments seems to be 10–20%. Mode 2 (OP + AG) predicts no overshoot. In Mode 4 (OP + AG + AMP deco.) this overshoot is small (5%), apparently too small to account for the entire PCr overshoot observed in experimental studies. On the other hand, the overshoot simulated in Mode 3 (OP + AG + down. reg.) (12%), in combined Mode 3 + 4 (18%), and, especially, in Mode 5 (OP + AG +parallel act.) (22%) is large enough to account for the magnitude of the PCr post-ischemic overshoot encountered in experimental studies.

Of course, strict quantitative comparison of computer simulations with experimental data is not possible. First, the real system is most probably a mixture of the different modes distinguished in this study, and not just one particular mode. Second, both the severity of hypoxia and the contribution of different processes/mechanisms can differ between different experimental systems.

Also the simulated time courses of PCr, ATP, P_i, and pH are generally similar to that observed in experiments (e.g., Ref. [5] is a good reference point), although, of course, there are some important differences between Modes 2, 3, 4 and 5. The first, intuitive inspection of such experimental data suggests that they do not correspond to any particular mode, but rather to a mixture of different modes (which is, in fact, what should be expected). For instance, down-regulation of ATP usage (decrease in RPP, measured explicitly) occurs in Ref. [5], which implies a contribution of Mode 3. [ATP] drops significantly (by approx. 50%), strongly suggesting AMP decomposition (contribution of Mode 4). Huge heart acidification (by 0.87 pH units) suggests a substantial role of anaerobic glycolysis (which is greatest in Mode 2) (this might suggest that the relative intensity of other processes is, in this case, smaller than in simulations; for instance the down-regulation of ATP usage observed in Ref. [5] is approximately 30% after 15 min of hypoxia, and not 50%, as in the simulation presented in Fig. 4). The kinetics (bi-phasic increase) and, especially, size of changes in [P_i] resemble that seen in Fig. 5 (Mode 4). The magnitude of the PCr overshoot (12%) suggests Mode 3 and, perhaps, Mode 5.

In combined Mode 3 + 4 the decrease in pH is rather small, 0.29 pH units. However, high values of both ATP usage down-regulation (by 50%) and AMP decomposition (leading to a decrease in [ATP] of approx. 50%) were applied in this simulation. It is likely these never occur in reality in such combination. If lower extents of these processes are assumed in combined Mode 3 + 4, the decrease in pH is larger whereas the PCr overshoot is smaller (not shown).

Assuming that different modes contribute to the observed pattern of the system behavior, it is worth analyzing the effect of particular processes/mechanisms on the kinetic properties of the system.

The positive and negative effects, usually related to benefits or harm, of particular processes/mechanisms on different system variables are summarized in Table 3. In the absence of any additional ATP source the blocking of oxidative phosphorylation by the low oxygen concentration would lead to complete collapse of the system: dramatic decrease in ATP turnover and thus heart work (compare Fig. 2), almost complete exhaustion of PCr and ATP, and huge increase in P_i and AMP (not shown). Recruitment of ATP supply by anaerobic glycolysis enables normoxic ATP turnover (and thus heart work) to be maintained (for a limited period of time), thus avoiding complete PCr and

Table 2 Comparison of computer simulations with experimental data

Variable value	Ref.						Simulations (Modes 2–5)
	[5]	[12]	[13]	[6]	[7]	[10]	
Hypoxia duration (min)	15	12	15	20	15	25	15
Decrease in [PCr] (to % of initial)	14	41	~10	~0	1^{c}–14^{b}	0^{b}–10^{c}	2–19
Time to 50% decrease of [PCr] (min)	1–2	–	–	1–2	<3	~1	1–2
Decrease in [ATP] (to % of initial)	~50	63	72	2^{ab}–$40^{a,\ c}$	66^{c}–74^{b}	50^{b}–60^{c}	46–98
Increase in [P_i] (times)	~7	2.8	2.3	–	–	5^{b}–8^{c}	3.2–5.8
Decrease in pH (pH units)	0.87	0.38	0.65	0.6^{ab}–$0.9^{a,\ c}$	0.8^{c}–1.0^{b}	0.6^{b}–0.8^{c}	0.36–0.81
Decrease in RPP, LVDP, or VO_2 (%)	~30	–	–	22^{b}–56^{c}	10^{c}–20^{b}	30–80	0–50
PCr overshoot (%)	~15	~15		10^{b}–20^{c}	3^{b}–9^{c}	15–25	0, 12, 5, 22^{d}

Variable values: changes in metabolite concentrations, pH, and heart work (expressed as RPP, LVDP, or VO_2) between normoxia and the end of hypoxia, the time for [PCr] to decrease to 50% of its initial value after the onset of hypoxia, and the size of the PCr overshoot taken from six literature reports are compared with those simulated in Modes 2–5 (involving anaerobic glycolysis, last column)

[a] After 15 min of hypoxia; [b] Preconditioned; [c] Not preconditioned; [d] in Modes 2, 3, 4, and 5

ATP exhaustion (beneficial effects), but at the cost of huge cytosol acidification (harmful effect). Down-regulation of ATP usage improves the stability of metabolite concentrations and pH (beneficial effects), but at the cost of lower heart work (harmful effect). AMP decomposition slightly delays cytosol acidification (beneficial effect), but worsens metabolite stability (harmful effect). Therefore, the presence of AMP decomposition during anoxia may indicate that avoiding extensive acidification is more vital for the function and survival of cardiac myocytes than maintaining relatively good metabolite stability. Generally, all the processes/mechanisms anaerobic glycolysis, ATP usage down-regulation, and AMP decomposition constitute a trade-off between improving some system properties and making other system properties worse. The only mechanism that seems to have, potentially, only beneficial effects is the hypothetical enhancement of the parallel activation of oxidative phosphorylation. This leads to a substantial improvement of pH stability and to a moderate improvement of metabolite stability. However, this mechanism cannot operate in anoxia.

The down-regulation of ATP usage, AMP decomposition, and parallel-activation enhancement lead to the phenomenon of the post-ischemic PCr overshoot that is frequently encountered in experimental studies [5–8, 12, 13]. No widely-accepted mechanical explanation of this phenomenon has been proposed in the literature. This theoretical study, on the other hand, offers three alternative or complementary explanations of the overshoot.

Kaplan and co-workers claimed to show that the post-ischemic PCr overshoot cannot be accounted for by decreased myocardial work (ATP usage down-regulation) during and, especially, after hypoxia [26]. The authors based their conclusion on the fact that the PCr/ATP ratio does not change significantly between different workloads. However, this interpretation may not be relevant in post-ischemic recovery. It was proposed that the stability of PCr, ATP, ADP, P_i, NADH, and other metabolite concentrations during low-to-high work transitions is caused by a perfectly balanced direct parallel activation of ATP usage, NADH production, and all oxidative phosphorylation complexes [15–18]. On the other hand, even if there is some parallel activation of oxidative phosphorylation in response to hypoxia, it certainly cannot ensure constant metabolite concentrations, as has been seen in many experiments. Therefore, the down-regulation can improve metabolite and pH stability, as shown in Fig. 4. Additionally, if myocardial work is still reduced during the post-hypoxic recovery, it will lead to the appearance of the PCr overshoot (Fig. 4).

The PCr overshoot is also frequently present in skeletal muscle during recovery after intense exercise [27, 28]. It has been postulated [22, 29] that this PCr recovery overshoot is caused by the slow decay during muscle recovery of the hypothetical parallel activation of oxidative phosphorylation complexes, which is turned on during muscle work. A possible role of this overshoot could be an activation, through the elevated [ATP]/[ADP_{free}] ratio, of different ATP/ADP-dependent processes in the cell, including RNA and protein synthesis [30], participating in repairing muscle cells after damage caused by such stress-generating factors as low pH, low ΔG_{ATP}, elevated muscle temperature, free radical production and mechanical stress [22, 28, 29].

The postulated explanation of the biochemical background of the PCr recovery overshoot in skeletal muscle [27, 28] is similar to the explanation of the post-ischemic PCr overshoot proposed within Mode 5 in this theoretical study. So what is new? Of course the passage from skeletal muscle to heart offers little novelty. Perhaps more

Table 3 Positive and negative effects of particular processes/mechanisms recruited in response to severe hypoxia (inhibition of oxidative phosphorylation, OP) in the heart on ATP turnover flux stability, metabolite stability, pH stability, and post-ischemic PCr overshoot

Process	ATP flux stability	Metabolite stability	pH stability	PCr overshoot
Effect of hypoxia with OP only	− − −	− − −	0	0
AG	+++	++	− − −	0
Down-reg.	−	+	++	++
AMP deco.	0	− −	++	+
Parallel act.	0	+	++	+++

OP, oxidative phosphorylation; AG, anaerobic glycolysis; down reg., down-regulation of ATP usage; AMP deco., AMP decomposition; parallel act., (increase in) parallel activation of oxidative phosphorylation; +++, dramatic positive effect; ++, strong positive effect; +, moderate positive effect; 0, no effect; − − −, dramatic negative effect; − −, strong negative effect; −, moderate negative effect

important is the factor that induces the parallel activation enhancement: it is postulated for the first time in this work that hypoxia can also lead to direct stimulation of oxidative phosphorylation. Kinetically, however, the most important difference is that, whereas in skeletal muscle the postulated slow decay of direct activation of oxidative phosphorylation after the termination of exercise remains the only existing explanation, in heart the situation is quite different. Here, two other mechanisms can contribute to the PCr post-ischemic overshoot. Although the effect of AMP decomposition seems to be too small to account for the entire magnitude of the overshoot, the ATP usage down-regulation seems to be able to generate, at least approximately, an appropriate magnitude of the overshoot. The effect is even greater if both processes are combined (Mode 3 + 4). This is not possible in skeletal muscle, because in this tissue ATP usage is already very low at rest/during recovery. It is even possible that in the heart the post-ischemic PCr overshoot can be explained without involving the (enhancement of the) parallel activation of oxidative phosphorylation. In such a case, the biochemical background of the PCr overshoot would be completely different in skeletal muscle during recovery and in heart after hypoxia.

The computer model used for simulations and the simulations themselves can be regarded as semi-quantitative only. First, the kinetic description of ATP and H^+ production by anaerobic glycolysis is very simple and phenomenological. On the other hand, this simple description was extracted [20] from the comprehensive model of glycolysis developed by Lambeth and Kushmerick [31]. Second, some model terms were adjusted to reproduce "typical" or average changes in the values of some variables. For instance, [ATP] can drop by 20–90% during approximately 15 min of hypoxia [5–13] (compare also Table 2) and the intensity of AMP decomposition assumed in the model results in a decrease of approximately 50%. Similarly, a typical decrease in the intensity of heart work during 15 min of hypoxia is usually in the range of 20–60% [5–9, 11] (compare also Table 2), and a decrease by 50% was assumed within the model. Also changes in the values of other variable, including pH, [PCr], and $[P_i]$, differ between various experiments, most probably reflecting differences in experimental systems, the contribution of different processes/mechanisms, and the severity of hypoxia. Finally, different magnitudes of the post-ischemic PCr overshoot are encountered.

The model assumes an instant decrease of the oxygen concentration from saturated to a very low value at the onset of ischemia. Of course, under real conditions this process takes some time. The model is also simplified in this respect in that it does not take into account the heterogeneity of oxygen distribution in the heart during ischemia. However, this does not seem to be a serious problem for the semi-quantitative simulations carried out in this study. Unlike in skeletal muscle, in the heart AMP is decomposed mostly by dephosphorylation to adenine, and not by deamination to IMP [32]. Nevertheless, the kinetics of these processes seem to be similar, at least semi-quantitatively, and can be approximated as a process with a constant rate over time. The same rate constant for proton efflux as in the model version for skeletal muscle was assumed. However, it is likely that proton efflux is slower in the ischemic heart, because in the absence of blood flow protons would quickly accumulate in the limited extracellular space. This would increase the cytosol acidification caused by anaerobic glycolysis.

The computer model used is relatively simple when compared with some other computer models of heart energetics [33–35]. However, more complex does not necessarily mean better. A model should be as complex as needed for a particular study, but no more. Simple models are much easier to understand and verify. They contain a much smaller number of adjustable, free values. Frequently, kinetic terms, for example K_m constants, measured in vitro cannot be extrapolated to in-vivo conditions. Therefore, the complexity of a model should be related to the problem that is to be resolved. In particular, the objective of this study was to test the effect of glycolysis, AMP decomposition, and ATP usage down-regulation on

the heart bioenergetic system during hypoxia, and not to analyze the detailed kinetics of these processes. Therefore, any more sophisticated kinetic description of these processes would offer no advantage. On the other hand, one should bear in mind all the time that computer models are at best only approximations of the reality with a limited range of applicability.

Other computer models have been used to study in silico the behavior of the bioenergetic system in the heart during hypoxia [33–35]. However, these studies were not devoted to analysis of the effect of anaerobic glycolysis, ATP usage down-regulation, AMP decomposition, and parallel activation on the system and of the biochemical background of the post-ischemic PCr overshoot. In fact, no overshoot was predicted by the model developed by Wu et al. [35]. Generally, these models are much more complicated in many respects than the model used in this study; in my opinion, however, they are not able to add anything important to the matters discussed in this article.

Ischemic preconditioning (IPC) in the heart [36] is caused by one or more periods of relatively short ischemia before the proper ischemia period studied. This results in improvement of metabolic (PCr, P_i) stability, less cytosol acidification, smaller down-regulation of ATP usage (manifested by a smaller decrease in the heart work, for instance RPP or LVDP), smaller AMP decomposition (manifested by a smaller decrease in ATP), and, sometimes, in intensification of the PCr post-ischemic overshoot [6, 7, 10, 11]. The biochemical mechanisms underlying this phenomenon are still not well understood. From among the processes/mechanisms considered in this study, intensification of anaerobic glycolysis cannot be the factor sought, because this would lead to a greater decrease in pH. A decrease in the glycolytic ATP supply alone would lessen cytosol acidification, but at the same time would reduce the ATP turnover flux (and thus cardiac output) and worsen metabolite stability. IPC is associated with a decrease in the down-regulation of ATP usage and AMP decomposition, as can be judged from the increased constancy of the heart work and [ATP], respectively. This by itself should lead to an increase in the cytosol acidification and, in the case of the decrease in the down-regulation, to a decrease in the PCr and P_i stability. Therefore, none of these mechanisms can be responsible for the effect of IPC on the heart bioenergetic system. For this reason, the only remaining candidate (from among the mechanisms considered in this study) is the postulated (hypothetical) enhancement of the parallel activation of particular oxidative phosphorylation complexes and the substrate dehydrogenation system (comprising TCA cycle, glycolysis, fatty acids β-oxidation, etc.). As was discussed above (compare also Table 3), this mechanism causes improvement of metabolite (PCr, ADP and P_i) stability, a decrease in cytosol acidification and an increase in the magnitude of the post-ischemic PCr overshoot. Of course, one cannot exclude mechanisms that are not analyzed explicitly in this article, for instance improvement of the oxygen supply to cardiac myocytes, an increase in the amount of myoglobin or its saturation with oxygen (and therefore increase of the oxygen stores in heart cells), etc. Generally, enhancement of parallel activation seems to be a logical, although speculative, explanation of some aspects of IPC.

The (still only hypothetical) idea of parallel activation (or each-step activation) of ATP usage and different steps of ATP supply, in particular essentially all oxidative phosphorylation complexes and the substrate dehydrogenation block (containing TCA cycle, glycolysis, fatty acids β-oxidation, etc.) [15–17, 19], originated on the basis of computer-aided theoretical studies on regulation of the adjustment of ATP production to ATP consumption during work transitions in the liver [18], skeletal muscle [15], and the heart [16, 17]. This mechanism is able to account for many different, apparently unrelated, system properties, e.g. the stability of metabolite concentrations [1–4] or the very short transition time for [PCr] and VO_2 during work transitions [37, 38] (reviewed in Ref. [19]). Although there is still no direct evidence for parallel activation in intact tissues, much indirect evidence is continuously accumulating. Experimental studies have shown that calcium ions can activate isolated heart [39] and skeletal muscle [40] mitochondria, at least under some conditions. It has been known for decades that Ca^{2+} activates three rate-controlling TCA cycle dehydrogenases: pyruvate dehydrogenase (PDH), isocitrate dehydrogenase (ICDH), and α-ketoglutarate dehydrogenase (KGDH) [41, 42]. It has also been proposed that mitochondrial calcium activates ATP synthase [43, 44]. Recently, Gellerich and co-workers discovered that external (in the intact cell—cytosolic) calcium activates aralar (glutamate/aspartate carrier), a component of the malate/aspartate shuttle [45]. It has been directly demonstrated experimentally, using the proportional activation approach, that adrenaline activates both ATP demand and supply in unpaced perfused rat heart [46]. The nature of the factor that causes the parallel direct activation of different enzymes in the cell bioenergetic system has not yet been fully identified. However, it has been proposed that the factor that activates particular transmembrane oxidative phosphorylation complexes and different cytosolic enzymes is cytosolic calcium [19]. It could act through, e.g., protein phosphorylation/dephosphorylation [15, 19]. Such a two-step mechanism (Ca^{2+} + protein phosphorylation/dephosphorylation) could be responsible for, e.g., the persistence of the parallel activation after the termination of skeletal muscle work (and the related ceasing of calcium oscillations) responsible for the PCr recovery overshoot. It could also account for the post-

ischemic PCr overshoot in the heart. This hypothesis is supported by the observation of calcium-mediated activation of pyruvate dehydrogenase (PDH), a component of the substrate dehydrogenation system, during ischemia [47].

The objective of this study was not to analyze the detailed kinetics of glycolysis, AMP decomposition, and down-regulation of ATP usage, but to test the quantitative effect of these processes on the heart bioenergetic system during hypoxia. Therefore, the simple kinetic description of these processes used in the computer model is sufficient for this purpose, as long as it reflects well (at least approximately) the "macroscopic" behavior of the system. However, for this reason, the model can be regarded as semi-quantitative and phenomenological only. On the other hand, a much more detailed kinetic description will be needed if one is interested in the particular mechanisms responsible for regulation of these processes.

To summarize, the effect of several mechanisms that can counteract the effect of substantial limitation of ATP supply by oxidative phosphorylation caused by severe hypoxia in the heart, namely a dramatic decrease in ATP turnover flux (and thus cardiac output), almost complete exhaustion of PCr and ATP, and a huge increase in P_i, ADP, and AMP, was estimated quantitatively. Strong stimulation of anaerobic glycolysis is able to maintain an unchanged ATP turnover flux, but at the cost of huge cytosol acidification, by up to 1.0 pH unit, and still with large changes in metabolite concentrations. ATP usage down-regulation compromises ATP turnover flux and cardiac output but improves the stability of metabolite concentrations and, especially, pH. AMP decomposition worsens metabolite stability but delays cytosol acidification. Finally, the hypothetical enhancement of the parallel activation of oxidative phosphorylation is able to increase oxidative ATP supply at the cost of glycolytic ATP supply (the overall ATP turnover remains unchanged), improve the stability of PCr, ADP, and P_i, and lessen the decrease in pH. However, this mechanism cannot operate in complete anoxia. For the first time, the genesis of the post-ischemic PCr overshoot in the heart is analyzed in detail and it is shown that all the last three mechanisms can lead to the appearance of this phenomenon. It is postulated that the parallel activation mechanism persisting for a long time after the termination of hypoxia is able to account for the effects of IPC on the kinetic properties of the bioenergetic system in the heart: increased stability of metabolite concentrations and pH, increase in the magnitude and duration of the PCr post-ischemic overshoot, and acceleration of system function recovery.

Generally, it is concluded that the heart uses different strategies to cope with the harmful effects of hypoxia on the bioenergetic systems of cardiac myocytes. All these strategies have advantages and disadvantages. Therefore, the relative contribution of these strategies during response of the heart to ischemia must be optimized in order to maximize benefits and minimize harm.

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