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REVIEW ARTICLE

The creatine kinase reaction: a simple reaction with functional complexity

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Abstract The classical role of PCr is seen as a reservoir of high-energy phosphates defending cellular ATP levels under anaerobic conditions, high rates of energy transfer or rapid fluctuations in energy requirement. Although the high concentration of PCr in glycolytic fast-twitch fibers supports the role of PCr as a buffer of ATP, the primary importance of the creatine kinase (CK) reaction may in fact be to counteract large increases in ADP, which could otherwise inhibit cellular ATPase-mediated systems. A primary role for CK in the maintenance of ADP homeostasis may explain why, in many conditions, there is an inverse relationship between PCr and muscle contractility but not between ATP and muscle contractility. The high rate of ATP hydrolysis during muscle contraction combined with restricted diffusion of ADP suggests that ADP concentration increases transiently during the contraction phase (ADP spikes) and that these are synchronized with the contraction. The presence of CK, structurally bound in close vicinity to the sites of ATP utilization, will reduce the amplitude and duration of the ADP spikes through PCr-mediated phosphotransfer. When PCr is reduced, the efficiency of CK as an ATP buffer will be reduced and the changes in ADP will become more prominent. The presence of ADP spikes is supported by the finding that other processes known to be activated by ADP (i.e. AMP deamination and glycolysis) are stimulated during exercise but not during anoxia, despite the same low global energy state. Breakdown of PCr is driven by increases in ADP above that depicted by the CK equilibrium and the current method to calculate ADPfree from the CK reaction in a contracting muscle is therefore questionable.

Keywords Adenine nucleotides · Creatine kinase equilibrium · Muscle energetics

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Introduction

Phosphorylcreatine (PCr) is mainly present in tissues with high rates of energy demand (i.e. skeletal muscle, cardiac muscle and brain). The higher concentration in fast-twitch fibers reflects the role of PCr in buffering cellular ATP during conditions of high rates of energy demand or oxygen deficiency. In addition to the well-established role as a temporal buffer of ATP, there is strong evidence that the CK reaction has an important role (a) as a spatial buffer of ATP (Wallimann et al. 1992), (b) in the control of oxidative phosphorylation (Bessman and Geiger 1981, Saks et al. 1976) and (c) as a buffer of protons.

This short report will highlight some questions related to the CK reaction: (a) What is the primary function of the CK reaction: to defend ATP concentration or to prevent increases in ADP? (b) Is the CK reaction at equilibrium in a contracting muscle and can the metabolically active form of ADP (ADPfree) be calculated from the CK equilibrium? and (c) Why is it that glycolysis cannot support resynthesis of PCr?

Role of PCr as a temporal buffer: to defend ATP content or to prevent increases in ADP

It has been known for a long time that a decline in PCr is related to a reduced force-generating capacity (Lundsgaard 1930), although the underlying mechanism is not clear. The muscle store of ATP is well protected by an efficient buffering by the CK reaction and the decrease in ATP is, in most cases, rather small even after intensive exercise to fatigue (<10%). However, since muscle content of ADP is at least 10 times lower than that of ATP, even a small decrease in ATP will lead to a large increase in ADP. Increases in ADP will reduce the energy release during ATP hydrolysis and may also reach concentrations that impair the ATPase reactions. As such, the primary function of the CK reaction in muscle energetics might be to prevent large increases in ADP rather than to defend the cellular ATP concentration.

The interior of the muscle cell is highly structurally organized and there is evidence that the diffusion of ADP is severely restricted (Wallimann et al. 1992), probably due to interaction with actin and myosin. This forms a system of subcellular compartments, where during muscle contraction, ADP increases transiently above that in the bulk phase. The contracting muscle will, therefore, be characterized by fluctuating ADP levels synchronized with the contraction at the sites of ATP utilization. The presence of isozymes of CK structurally bound in close vicinity to the sites of ATP utilization (myosine-ATPase, Ca-ATPase and Na^+/K^+-ATPase) will reduce the amplitude and duration of the transient increases in ADP (ADP spikes) through PCr-mediated phosphotransfer. During conditions of reduced PCr levels the CK buffering will be less efficient or absent and the amplitude and duration of ADP spikes will become larger (Fig. 1), eventually reaching a concentration that impairs the contraction process. There is evidence that the presence of PCr is essential for maintaining adequate function of the contractile system and that this cannot be replaced by maintained high levels of ATP (for references see Wallimann et al. 1992).

Studies with time-resolved magnetic resonance spectroscopy (MRS) have shown that PCr changes during the contraction cycle of a twitch (Chung et al. 1998). Within 16 ms after initiation of the twitch PCr decreased by 11.3% followed by a rapid (within 40 ms) reversal back to the steady state value. The changes in PCr correspond to changes in ADP occurring in the opposite direction. These findings demonstrate that there are rapid fluctuations in energy state, synchronized with the contraction, which occur within milliseconds. The measured decrease in PCr (3 μmol/g/twitch) is 17 times higher than the estimated energy turnover in the whole muscle and suggests that the changes in PCr observed with MRS occurred in localized cellular regions different from the cytosolic bulk phase. To drive the CK reaction in the forward direction (PCr breakdown) there is a kinetic necessity that ADP increases above that predicted from the CK equilibrium. Given the kinetic constraints of the CK reaction, it was calculated that ADP must increase 14-fold to account for the rapid forward CK flux rate during contraction (Chung et al. 1998). This is much higher than the twofold increase in ADP calculated to occur in the bulk phase during the actual contraction (Meyer and Foley 1996) and is consistent with the idea of contraction-induced ADP spikes occurring in the vicinity of ATP utilization.

Further evidence for contraction-induced increases in ADP above the steady-state level comes from measurements of the product of adenine nucleotide catabolism. Increases in ADP will, due to the high activity of adenylate kinase (AK), increase AMP concentration, which will activate AMP deaminase and result in deamination of AMP to inosine monophosphate (IMP). The AMP deaminase reaction is irreversible, which is in contrast to the CK and AK reactions. The IMP level will, therefore, show the flux rate integrated over the exercise period and will reflect the magnitude of increases in AMP and ADP. The concentration of IMP is very low in non-active muscle and after low-intensity exercise but shows a pronounced increase after exhaustive exercise (Sahlin et al. 1978). Recovery of muscle energetics is dependent upon oxygen supply. When

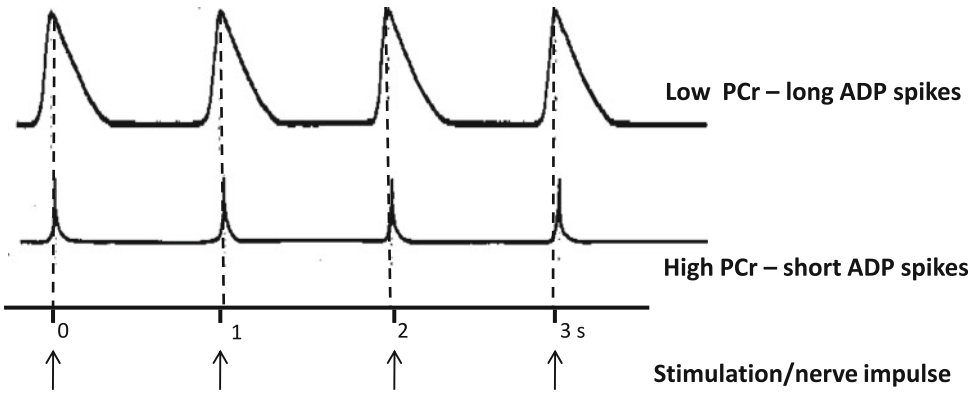

Fig. 1 Schematic graph showing hypothetical changes in ADP occurring locally in the cell close to the site of ATP utilization

blood flow is occluded after exercise PCr is maintained at a low level. However, despite a low PCr (reflecting increased steady-state levels of ADP and AMP) AMP deamination is blunted when contraction is terminated (Sahlin et al. 1990). IMP formation is thus closely related to the contraction phase and this is consistent with the idea that large increases in ADP and AMP occur during the actual contraction.

CK equilibrium and ADPfree

The concentration of adenine nucleotides (ATP, ADP and AMP) has a crucial role in the control of the ATP yield and the ATP-utilizing reactions (Atkinson 1968). Total cellular content of ADP and AMP is less than 1 and 0.1 mM, respectively, which is about 10 and 1% of the ATP content. Furthermore, the major part of ADP and AMP is considered to be bound to proteins or otherwise sequestered. The concentrations of the metabolically active form of ADP (ADPfree) and AMP (AMPfree) are therefore very low. A major challenge in the understanding of the control of muscle energetics is to determine the concentrations of the metabolically active forms of ADP and AMP during different conditions. Veech et al. (1979) compared the mass-action ratios of enzymatic reactions known to be close to equilibrium (CK, AK and the combined glyceraldehyde-3-phosphate dehydrogenase/3-phosphoglycerate kinase) with that in vitro. They concluded that a large part of ADP was not available to the cytosolic enzymes and that the concentration was about 20-fold lower than the whole tissue content. The results from this work and the accompanying paper (Lawson and Veech 1979) form the basis for the current convention to calculate ADPfree from the CK equilibrium and subsequently AMPfree from the AK equilibrium. Using this procedure it can be calculated that the concentrations of ADPfree and AMPfree, in human skeletal muscle at rest, are only 0.02 and 0.00007 mM, which corresponds to 2 and 0.2% of the total tissue content determined after acid extraction. Although being an established method in muscle energetics, calculation of ADPfree from the CK reaction has some limitations, which under certain conditions may give erroneous results.

MRS measures only phosphorous metabolites, which are free in the cytosol (i.e. the metabolic active forms and will not detect phosphorous metabolites bound to proteins). The concentrations of ADPfree, however, are too low and appear superimposed on the ATP signal, meaning that they cannot normally be observed with MRS. However, in experiments with transgenic mice with ablated AK enzyme, muscle contraction elicited a large increase in ADP, which was visible with MRS (Hancock et al. 2005a). The observed concentration of ADP was 1.7 mM and was similar to the increase in total muscle content of ADP as measured after acid extraction. In contrast, calculation of ADPfree from the CK equilibrium, using the data presented by Hancock (Hancock et al. 2005b), gives a concentration of 0.04 mM or only about 2% of that actually measured. The large difference between measured ADPfree (MRS) and calculated ADP could possibly be due to the fact that MRS only measures ADP in a localized metabolically active region. Alternatively, calculation of ADPfree from the CK equilibrium in a contracting muscle is not correct.

Calculations of ADPfree from the CK equilibrium are based upon the assumptions that (a) $[Mg^{2+}]$ is maintained at 1 mM, (b) total tissue contents of ATP, PCr and Cr are available to the CK reaction and (c) the CK reaction is near equilibrium. As previously discussed, the assumption of near-equilibrium conditions can be questioned during conditions of rapid changes in the PCr/Cr system, such as during intensive muscle contraction. Kinetic calculations show that ADPfree must be 2–14 times higher than that predicted from the CK equilibrium during contraction (Chung et al. 1998; Meyer and Foley 1996). Considering the critical importance of the adenine nucleotides in metabolic regulation, further studies with alternative techniques are required to determine ADPfree in a contracting muscle.

PCr resynthesis

During the recovery from exercise there is a rapid resynthesis of PCr back to or above the pre-exercise value. The time course of PCr resynthesis has two phases, where the first dominating phase has a half-time of about 22 s and the latter phase about 3 min (Harris et al. 1976). There is evidence that the latter (slower) phase is dependent on the rate of pH recovery and is absent during conditions without lactic acidosis (McMahon and Jenkins 2002). PCr resynthesis is dependent on ATP provision, which theoretically could be provided either from oxidative phosphorylation or substrate phosphorylation (i.e. glycolysis). However, experiments in humans have shown that resynthesis of PCr is completely blocked when oxygen supply to the muscle is prevented by local circulatory occlusion (Harris et al. 1976; Quistorff et al. 1993; Dawson et al. 1980). When muscle samples, taken after exhaustive exercise that resulted in depleted PCr stores, were incubated for 15 min in anaerobic atmosphere (N_2 + 5% CO_2), PCr remained at a low level without any signs of resynthesis (Sahlin et al. 1979). In contrast, when muscle samples were incubated in the presence of oxygen (O_2 + 5%CO_2), PCr was restored back

to the basal level (Sahlin et al. 1979). These experiments clearly demonstrate that resynthesis of PCr can only occur with ATP derived from oxidative phosphorylation and that glycolysis is unable to provide the required ATP. The initial rate of PCr resynthesis was suggested to be a measure of the maximal rate of oxidative phosphorylation and thus of the muscle oxidative potential (Hultman et al. 1981). Later studies have confirmed that the rate of PCr resynthesis is correlated with muscle oxidative potential (Takahashi et al. 1995).

The absence of PCr resynthesis during anaerobic conditions demonstrates that glycolysis is unable to support PCr resynthesis. Since the prevailing low PCr reflects increased levels of ADP and AMP it was argued that glycolysis is activated by factors other than the energy state and that the close association between glycolysis and contraction implies that glycolysis is activated by increases in cytosolic Ca^{2+} (Conley et al. 1997; Wilkie et al. 1984). In a recent experiment, this hypothesis was tested by pharmacological blockade of the cross-bridge cycle with BTS, which reduced energy turnover by approximately 40% (Ortenblad et al. 2009) without changes in Ca^{2+} transients. The results from this study demonstrated that the control of glycolysis was indeed related to energy state and are contrary to the hypothesis that Ca^{2+} is the major trigger of glycolysis. The inability of substrate phosphorylation to support PCr resynthesis during anaerobic conditions may relate to the fact that activation of glycolysis requires more pronounced increases in ADP and AMP than the steady-state levels depicted by the CK and AK equilibrium. The hypothesis of large increases in ADP and AMP occurring at the sites of ATP utilization during the actual contraction could be the explanation for the close association between the contraction process and activation of glycolysis.

Concluding remarks

The high rate of energy release during exercise results in increases in ADP above that calculated from the CK equilibrium. The restricted diffusion of ADP and the presence of CK at the ATP-utilizing sites suggest that contraction-induced ADP spikes occur in localized cellular regions. The presence of ADP spikes in contracting muscle is supported by measurements with time-resolved MRS and by the observed association between contraction and ADP-dependent processes such as AMP deamination and glycolysis. Using the CK equilibrium to calculate ADPfree will not provide an accurate representation of the rapid fluctuation in energy metabolism that occurs in a contracting muscle.

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