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Review

Metabolic control and metabolic capacity: two aspects of creatine kinase functioning in the cells

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''The fundament upon which all our knowledge and learning rests is the inexplicable. It is to this that every explanation, through few or many intermediate stages, leads''.

Arthur Schopenhauer, ''Parerga and Paralipomena'', 1851

1. Summary

In this short review, the merits and limits of three theoretical concepts explaining the functional role of the creatine kinase system in muscle and brain cells are analysed. In addition to the usual concept of an energy buffer system and the recently proposed metabolic capacity theory (Sweeney, H.L. (1994) Med. Sci. Sports Exerc. 26, 30–36), it is proposed that coupled creatine kinase systems are involved in effective metabolic regulation of energy fluxes and oxidative phosphorylation, beside their energy transfer function. This aspect of the system is considered on the basis of metabolic control analysis. It is shown by using the results of mathematical modelling that, due to amplification of ADP fluxes from the cytoplasm by the mechanism of metabolic channelling, coupled mitochondrial creatine kinase may exert a flux control coefficient significantly exceeding 1.

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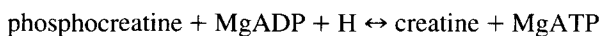
2. Introduction

In studies of cellular bioenergetics, the complex nature of interactions between different cellular components and their influence on each other – functional coupling of different processes by a mechanism of metabolic channelling, leading to the phenomenon of metabolic compartmentation – is becoming more and more obvious. This conforms to the understanding that to elucidate the mechanisms of cellular regulation, the complex interactions between structural and functional units should be accounted for. New developments in studies of creatine kinase systems may well illustrate this conclusion.

The creatine kinase reaction was discovered in muscle extracts by Karl Lohman in 1934 in Germany [1]. This reaction:



phosphocreatine + MgADP + H ↔ creatine + MgATP

is catalyzed by different isoenzymes of creatine kinase (CK), which are present in numerous tissues (for recent reviews see Refs. [2,3]) and play important roles in energy metabolism in heart, skeletal muscle, brain and smooth muscle. These isoenzymes are kinetically very similar but differ in their capacity to associate with subcellular organelles or protein structures [2,3]. The CK isoenzymes, brain form BB, muscle form MM and two mitochondrial forms, sarcomeric (expressed mostly in heart and skeletal muscle and probably in some brain cells such as Purkinje neurons) and ubiquitous (expressed in many tissues) MiCK, are encoded by different genes, the homology between BB and MM being 77–82%, and that between MM or BB and MiCK isoenzymes 60–65% [4,5]. The phylogenetic (evolutionary) tree constructed on the basis of protein (or nucleic acid) sequences shows that, during evolution, a first gene duplication event resulted in primordial cytoplasmic and mitochondrial CK isoenzymes, gene duplications giving rise to the multiple cytoplasmic and mitochondrial isoenzymes occurring at a later stage during evolution [4]. Why was that – the appearance of MiCK being different from the others – necessary? what was and still is the significance of this event for the cell's life? To answer these questions, we have to understand the functional aspect of the problem – what is the function of CK in the cells? Three possible answers to these questions can be found in the literature.

2.1. *Creatine kinase: energy buffer system*

It is an energy buffer function of the equilibrium creatine kinase reaction which is usually ascribed to the system, consisting of creatine kinase, creatine (Cr) and phosphocreatine (PCr) and related to the ATP-ADP system. According to this concept, the enzyme simply serves the purpose of maintaining an adequate ATP/ADP ratio in the cells with increased energy demand [6,7]. Historically, this role was ascribed to the creatine kinase system after classical works by the Davies group showing that one observes ATP splitting in the contracting muscle cells if creatine kinase is inhibited [8,9]. Since that time, it has been assumed that ATP produced in phosphorylation reactions coupled to substrate oxidation in mitochondria or glycolysis freely and rapidly diffuses to all sites of its utilization to be used in ATPase reactions to cover all cellular requirements in energy. Accordingly, ADP is taken to diffuse rapidly back for rephosphorylation. Thus, all energy requirements of the cell can, according to this concept, be explained on the basis of the biochemistry of adenine nucleotides. Concerning phosphocreatine and also phosphoarginine (in the past both were called 'phosphagens' [9]), it was assumed that they serve only as a store of energy, being produced via equilibrium creatine (or arginine) kinase reactions from ATP produced in some excess with respect to cellular needs of energy. These 'energy stores' were considered to be used only in times of significant fluctuations in energy need to restore the necessary amount of ATP via the same equilibrium kinase reactions, and therefore, these compounds were also called temporary energy buffers. For this function, one isoenzyme of creatine kinase functioning in equilibrium is sufficient. The basic principle of this concept is the use of the creatine kinase equilibrium equation. The apparent equilibrium constant of the CK reaction at pH 7.0, $K'_{eq} = [ATP] \times [Cr]/[ADP] \times [PCr]$ is 166, and if the reaction is close to equilibrium, the ATP/ADP ratio exceeds 100 under physiological conditions in muscle cells [10,11]. A practical aspect of this concept is that it allows one to calculate the free cytoplasmic ADP concentration in the cells – around 50 μM, which cannot be measured either biochemically or by NMR, and this aspect is widely exploited in practically all modern metabolic studies. There is good evidence that in the cytoplasmic compartment of the muscle cells creatine kinase is rather close to equilibrium [12]. This compartment contains mostly creatine kinase MM isoenzyme; in fast skeletal muscle its activity is about 5–7-times greater than in the heart, slow skeletal muscle being somewhere between them [13–15]. Magnetization transfer studies using the phosphorus NMR technique allowed the determination of the rate constants and fluxes in both direction; they were found to be equal to each other and to exceed the total energy flux – steady-state rates of ATP production, energy transfer and utilization – almost by an order of magnitude, the latter evidence conforming to the cytoplasmic equilibrium of creatine kinase substrates [16,17].

This simple and useful concept which is employed in most studies of cellular metabolism may help to clarify some events (concentration values, fluxes, etc.) in the cytoplasmic compartment, nevertheless, it still does not provide a satisfactory explanation for the existence of different creatine kinase isoenzymes. It is important to note here again that all of them – mitochondrial isoenzyme, MM or BB – have very similar kinetic and thermody-

namic characteristics, since they all have the same conserved structure of the active center [4], and in the cells the major difference is in their intracellular localisation [2,3]. The simple temporary energy buffer concept of creatine kinase is not sufficient to illuminate this phenomenon, and thus lacks an explanation for the appearance of MiCK during evolution. One rather mysterious argument often used by faithful proponents of this simple theory is that, since an equilibrium state in the cytoplasmic compartment conforms to the buffer role of creatine kinase, it precludes any other approaches.

2.2. Metabolic capacity system

However, simple and already often complemented quantitative analysis of the equilibrium equation using a value for the equilibrium constant of 166 showed that in fact about 99% of the energy flux via equilibrium creatine kinase is represented by that of phosphocreatine via a mechanism of facilitated diffusion [18]. This modifies the concept of the simple temporary energy buffer role of creatine kinase into that of a metabolic capacity system with a new additional property – spatial buffering [18–20]. This theory places the equilibrium creatine kinase from the sidewalks onto the central pathway between energy production and utilization and is well illustrated by analogy with an electrical circuit with capacity between resistance (ATPases) and battery (mitochondria), and from this analogy the theory derived its name [19]. It takes into consideration the structural organization of muscle cells – that cytoplasmic space containing a significant amount of MM creatine kinase is between mitochondria and myofibrils in these cells. According to this concept, mitochondria release ATP into the cytoplasm but then the equilibrium creatine kinase transfers (by a mechanism of facilitated diffusion of high-energy phosphate bonds) a significant proportion of the energy through the cytoplasm as a flux of phosphocreatine (spatial energy buffering – maintaining local ATP/ADP ratios). This concept is also entirely based on the consideration of equilibrium creatine kinase in homogeneous medium in one or several cellular compartments, and therefore it also preserves the energy store function (temporary buffering) for phosphocreatine. A combination of facilitated diffusion of high-energy phosphate bonds in the cytoplasm with the energy store function confers on the creatine kinase system the property of metabolic capacity [19,20]. This aspect of the function of the creatine kinase system was recently analysed in detail by Sweeney [20]. The main advantage of this system is that is allows very rapid but short-burst energy use at the extended low rate of energy production [20]. Metabolic capacity of the creatine kinase system also allows equal distribution of energy inside cells even if its production is localized (mitochondrial clustering around capillaries [21]). This seems to be the most satisfying explanation for the energetics of fast skeletal muscles with their mainly glycolytic energy production and oxygen debt phenomenon. This theory explains the localization of CK isoenzymes in cellular structures by the necessity of creating equilibrium systems to maintain constant ATP/ADP ratios locally [18,20]. However, a very interesting biological phenomenon – a decrease in total cellular creatine kinase activity and total creatine content with elevation of total energy demand (as seen from comparison of fast, slow and cardiac muscle) [2,3,13–15] when one might expect an increase of capacity – still awaits its explanation within the framework of this theory. Also, this high capacity system, delaying the response of mitochondria to rapid increase in energy demand, temporarily uncouples energy production from its utilization – a property not very compatible with high rates of energy utilization and fast work transitions as occurs in the heart. The explanation is most probably that in these cells, the capacity of the CK system is decreased but this is compensated for by creation of new systems of higher efficiency of regulation – coupled creatine kinases with the new quality of strong metabolic control. In this work we wish to emphasize this 'new' function of the CK system.

2.3. Energy transfer and metabolic control system

The third concept of the physiological role of the creatine kinase system has been developed for more than 40 years, starting with the pioneering works of Bessmann et al. (see Refs. [2,3,22,23] for reviews). It considers, in addition to facilitated energy transport by equilibrium creatine kinase in the cytoplasmic compartment, direct functional coupling of MiCK with oxidative phosphorylation via adenine nucleotide translocase in mitochondria and MM creatine kinase with MgATPases in myofibrils and on cellular membranes [2,3,22,23]. This is the concept of a phosphocreatine 'shuttle' [22] or 'circuit' [2,3] which is based on the compartmentation of creatine kinase isoenzymes and adenine nucleotides in the muscle and brain cells. Biochemical and physiological evidence for these coupled systems is abundant and has been reviewed extensively [2,3,22,23,25,26,31]; here we analyse the meaning of this system for the regulation of cell function.

If energy demand is increased and energy is used permanently in contraction cycles, a more direct and efficient way than the capacity system for the control of energy production and supply in response to a rapid increase in workload can be achieved simply by using the benefits of organization. Mitochondria in these cells become increasingly numerous and in heart cells they occupy up to 30–40% of the cell volume [24]. Increased energy demand in slow skeletal muscles or its permanent use in cardiac muscle requires fine regulation of its production and effective intracellular transfer, and most probably the creatine kinase systems were developed during evolution both in mitochondria and in myofibrils as well as at the cellular membranes to fulfil such requirements [2,3,22,23].

While the total activity of creatine kinase decreases in the following order: fast skeletal – slow skeletal – cardiac muscle, the activity of mitochondrial creatine kinase increases in the same order and its share reaches 30–40% of total enzyme activity in the cardiac muscle: these two parameters of the creatine kinase system are inversely related to each other [13–15]. The mitochondrial creatine kinase isoenzyme is located at the outer surface of the inner mitochondrial membrane all along the cristae membrane and is mostly bound to the cardiolipin domain which surrounds the adenine nucleotide translocase [23,25]. It may also form complexes with porin pores in the outer mitochondrial membrane [2]. These structural complexes are located between the cytoplasm and mitochondrial inner membrane-matrix space where oxidative phosphorylation takes place. Therefore, they are in fact in position to dictate the course of events and to control local ADP and ATP concentrations in the intermembrane space of mitochondria according to the mechanism of functional coupling, leaving little place for the global use of Michaelis-Menten kinetics and of calculated average cytoplasmic ADP concentration for explanation of the regulation of respiration and energy fluxes in general. The phenomenon of functional coupling has been described in multiple investigations (reviewed in Ref. [23]) and means substract-product, or metabolic channelling between translocase and MiCK due to their very close spatial proximity: translocase provides ATP from the matrix to the microenvironment of MiCK and takes back the ADP produced, in the presence of creatine, simultaneously with phosphocreatine. This ADP is in turn taken up by translocase back into the matrix for rephosphorylation to start the new cycle. This coupled cycle is repeated many times and increases the turnover of adenine nucleotides, ending up in production of large amounts of phosphocreatine with a PCr/O ratio close to 3 [15,26]. In this very precisely organized system, the maximal activity of mitochondrial creatine kinase is equal to the rate of ATP production and translocation, and thus, to the total energy flux [15,23,26]. This means that all molecules of creatine kinase work hard and in one direction, leaving no place for a relaxed equilibrium state; however, as a result of this fine organization the work is done very effectively, using a limited amount of enzymes at the right place. If we look from the cytoplasmic side, the entry of even a very small amount of ADP into mitochondria is enough for acceleration of respiration, since the functionally coupled system is in fact a powerful amplifier for the regulatory action of ADP. Recent studies have revealed that this may be the most important property of coupled MiCK, since the outer mitochondrial membrane porin channels [27] appear to have a low permeability for ADP in cells in vivo, in contrast to what is known for isolated mitochondria in vitro [28,29]. Indeed, this kind of specifically restricted ADP diffusion can be easily and directly shown in permeabilized cardiomyocytes or skinned muscle fibers, as demonstrated in

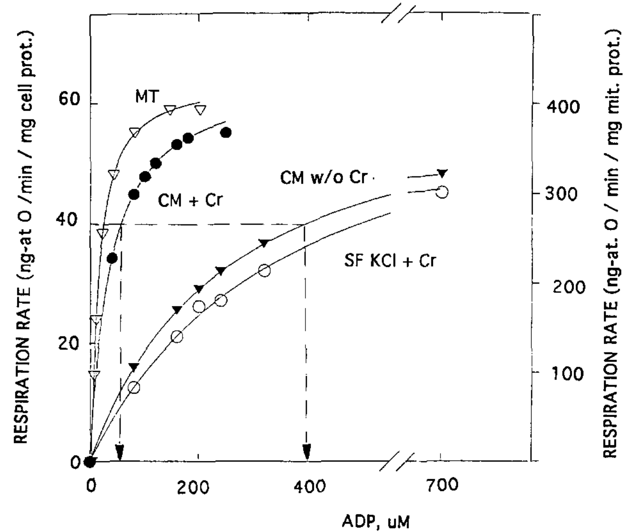


Fig. 1. The problems with regulation of respiration in cardiac cells in vivo: in contrast to isolated mitochondria (MT) which are rapidly activated when the ADP concentration in the medium increases (apparent K_m 17 μM), in skinned cardiomyocytes (CM) or cardiac fibers (SF) without creatine (w/o Cr) the respiration rate increases very slowly with elevation of ADP concentration and almost millimolar concentration of ADP is needed for maximal activation of respiration (apparent K_m around 300 μM). In the presence of 25 mM creatine, when mitochondrial creatine kinase which is attached to the outer surface of the inner mitochondrial membrane produces ADP and channels it directly to translocase, the apparent K_m for ADP is decreased to 40–70 μM and only a small amount of ADP is needed to activate the coupled reactions in which adenine nucleotide turnover is increased by almost an order of magnitude. For example, to reach a respiration rate of 40 ngatom O/min per mg cell protein, in the case of CM without creatine, the ADP concentration should attain a value of 400 μM, but in the presence of 25 mM creatine the range of necessary ADP concentrations is decreased to 50–60 μM (see dashed lines and arrows). This amplifying effect of coupled creatine kinase reaction on ADP regulation of cellular respiration in vivo is lost if creatine kinase is detached from the membrane in 125 mM KCl solution (SF KCl + Cr). (From Ref. [29] with permission.)

Fig. 1. In contrast to isolated mitochondria, the apparent K_m for ADP in regulation of respiration in permeabilized cells is more than an order of magnitude greater and equal to 300–400 μM, but is decreased to 40–70 μM if the creatine kinase reaction is activated and ADP produced in the intermembrane space in the coupled reactions; this means that high rates of respiration are maintained at low ADP concentrations, in spite of the low permeability of the outer membrane for this substrate in vivo – this is the amplification effect of the MiCK reaction on ADP regulation of respiration. Remarkably, and this is very important, detachment of the enzyme from the mitochondrial membrane results in complete loss of this kind of control [30,31] in spite of the enzyme remaining in the intermembrane space – the direct metabolic channelling, and therefore functional coupling, is lost (Fig. 1). This shows that interaction between translocase and creatine kinase is indeed based on the direct channelling of ATP and ADP between their binding (active) sites and not on the accumu-

lation of these substrates in the intermembrane space, which, however, may influence the reaction especially if the outer mitochondrial membrane permeability is limited [28]. Additionally, in cells in vivo the intermembrane space could be very much reduced as compared to in vivo conditions, and the outer membrane may be in continuous contact with the inner membrane [23,27].

Symmetrical reaction in another direction – local ATP resynthesis – occurs in all energy-consuming systems catalysed by coupled MM-CK [23,32]. For contraction, the rate of ADP removal is essential, since it is an active component of the contraction cycle; its dissociation from myosin heads is related to the detachment of crossbridges and, if it accumulates in myofibrils, it slows down the process of crossbridge detachment, decreasing the rate of sliding of crossbridge-myosin heads – along the thin filaments [33,34] and thus the rate of force development, finally resulting in elevation of resting tension. Rapid removal of this ADP is the task of MM-CK localised in myofibrils in M-line and inside the I-band of sarcomeres. This is most probably not an equilibrium but an almost unidirectional steady-state process due to the favourable kinetic properties of MM-CK, and also occurs inside the myofibrillar compartment due to some kind of metabolic channelling [32]. Interestingly, the removal of ADP from crossbridges may to some extent be effectively performed by the glycolytic complex located in the I-band [35,36]. Transgenic animal studies by using a series of mouse mutants with subnormal MM-CK expression confirmed that the ability of muscles to perform burst activity correlates with the level of MM-CK expression [37,38]. In these studies one new and interesting observation was made: when enzyme activity was decreased to 34% of normal, phosphorus NMR spectroscopy failed to show any flux in the CK system, but phosphocreatine was consumed in contractions [37,38]. Most probably, this directly shows that structurally bound creatine kinases in myofibrils and subcellular membranes (up to 30% of total) are functionally connected to ATP pools in local microcompartments which may be NMR invisible due to interaction with proteins. A similar conclusion was drawn by Suzuki et al. who discovered a fraction of ATP with a long (14 s) T_1, probably due to interaction with proteins in rat heart, which represented about 30% of the total ATP content [39]. These conclusions are in good concord with the recent interpretation of these phenomena by Wallimann [40].

Thus, in addition to the metabolic capacity system – equilibrium creatine kinase in cytoplasm – we have the coupled creatine kinase isoenzymes functioning in steady state, via a mechanism of functional coupling (metabolic channelling), but in different directions depending on localization. Metabolic channelling is very often related to the existence of microcompartments, as illustrated by creatine kinase functioning in myofibrils and probably in mitochondria [23]. Moreover, one of the advantages (besides decreasing the transient time of consecutive reactions and achieving the goals by using small numbers of enzymes) of the systems of metabolic channelling is that it has significantly increased flux control properties. Metabolic capacity systems are built to slow down and extend in time the metabolic response to load [18–20]. In the case of high and permanent fluctuation of energy demand this is not a useful property for rapid activation of mitochondrial energy production. Modern theories of metabolic control analysis describe the control of fluxes quantitatively by flux control coefficients, and one of its basic theorems states that the sum of control coefficients does not exceed 1 in homogeneous systems ([41], see Appendix). Kholodenko et al. have developed this theory further, showing that in the systems of metabolic channelling the sum of flux control coefficients significantly exceeds 1 [42–45], and we may conclude that such systems are controlled much more effectively than simple, homogeneous metabolic capacity systems. This means that the coupled creatine kinase systems acquire a new property – that of effective metabolic control, already illustrated in Fig. 1. Aliev and Saks have developed a mathematical model of coupled creatine kinase reaction in mitochondria based on the probability approach ([46,47], see Appendix). Fig. 2 reproduces one of these interesting results: in the model of the fully coupled creatine kinase reaction its activity was taken to be inhibited by 1%, and that decreased the rate of coupled oxidative phosphorylation by more than 8% as a result of decreased turnover of adenine nucleotides at very low ATP concentration, when the 'uncoupled' creatine kinase reaction is practically impossible [48]. This effect is consistent with the results shown in Fig. 1 which directly demonstrate increased turnover (about 5–8 times) of ATP-ADP in mitochondria by functionally coupled CK reaction. With elevation of the ATP concentration this flux control coefficient decreases but remains above 1 (Fig. 2).

This means that in fully coupled systems the flux control coefficient may have a value higher than 8 and thus exceeds the value of 1 of homogeneous systems very significantly.

3. Conclusions

It is an interesting and even entertaining thought that all three concepts described are true, depending on what we want to know about creatine kinases. The first one may still be completely sufficient to explain the cell metabolism for the period of prenatal development when one observes the appearance of soluble non-coupled creatine kinases, first B-type that later is switched in muscles to M-type subunits [5]. Mitochondrial creatine kinase coded by two separate genes in mammalian hearts appears only during the perinatal period in parallel with cell maturation when a several-fold increase in workload takes place, obviously to match the requirements for increased energy fluxes and

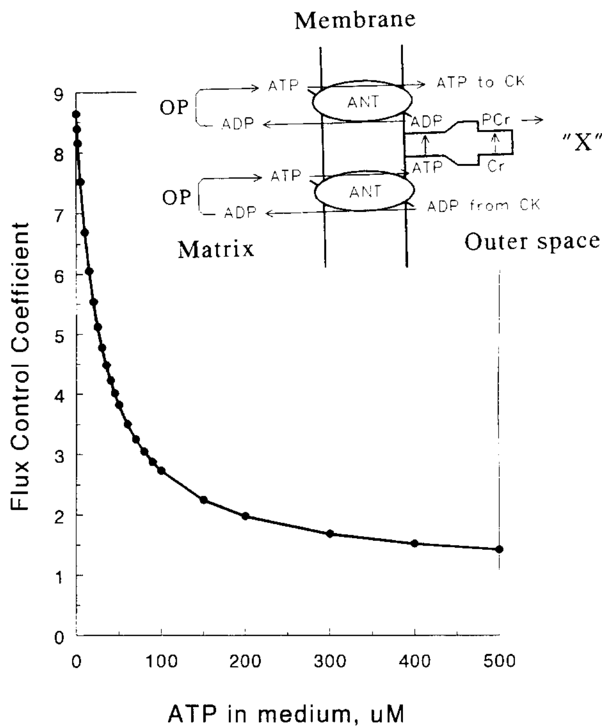


Fig. 2. One of the results of mathematical modelling of coupled creatine kinase reaction in heart mitochondria performed by Aliev and Saks [46,48]. The mathematical model is based on the probability approach and on the assumption of direct transfer of ATP and ADP molecules between mitochondrial creatine kinase and translocase, as shown by the scheme in the upper right corner (for further explanations see Appendix). Flux control coefficient: calculated change in coupled creatine kinase-oxidative phosphorylation (OP) reactions when 1% of creatine kinase is taken to be inactivated. Creatine concentration was assumed to be 25 mM and ATP concentration change as shown. At high ATP concentration the flux control coefficient exceeds 1 and when the ATP concentration in the medium decreases and approaches zero, the flux control coefficient approaches values exceeding 8! [48]. This is explained by high turnover of ATP and ADP in coupled reactions (see Fig. 1). The dependence of this parameter on ATP concentration is explained by the fact that the lower the ATP concentration, the lower the rate of its use in 'non-coupled' CK reaction and the higher the share of ADP supply in coupled CK reaction [48]. Of course, at zero ATP even coupled reactions stop. (From Ref. [48] with permission.)

their fine control [49]. For fast skeletal muscle, which contains only a small amount of mitochondria and a very large amount of soluble cytoplasmic creatine kinase, the metabolic capacity theory for creatine kinase may be a satisfying explanation. In mature mammalian hearts, slow twitch skeletal muscle and most probably in the brain, the metabolic capacity theory, however, is insufficient, because it gives only one, cytoplasmic part of the story. In these and many other tissues [2,23] coupled systems with metabolic control properties should be taken into consideration. In addition to knowledge of the system, this may be a very useful concept in explaining many important phenomena. For example, recently the Schultheiss group in Germany obtained good evidence for the contribution by the destruction of the mitochondrial coupled creatine kinase system to acute ischemic heart failure [50]. Similar findings were made for cardiomyopathy [51]. This may lead to an important conclusion that only minor destruction of fine structural organization may lead to severe pathology of the cell.

Thus, all three functions of the creatine kinase system are integrated in cells and cannot easily be dissected from each other. The temporary energy buffering role of this system was understood since the studies of Cain and Davies [8]. The metabolic capacity function was deduced from quantitative analysis of the equilibrium creatine kinase reaction first by Nagle in 1970 [52] and significantly later by Meyer and Sweeney [18–20]. The third approach takes into account the highly organized structure of the cell interior and the existence of multiple compartments and direct metabolic channelling in the cells. This role of the creatine kinase system in energy transfer and metabolic control has been confirmed by numerous biochemical and physiological studies of coupled creatine kinase systems in vitro reviewed in [2,3,22,23,29,32]. Further, significant functional and structural changes in muscles induced in energy-deficient states due to decreased level of phosphocreatine and creatine in experiments with its analogs [23], due to alterations in creatine kinase isoenzyme expression in pathologies such as dilated cardiomyopathy [51] or in experiments with 'knock-out' of these isoenzymes in transgenic animals [37,38,40], are consistent with this concept of the central role of the creatine kinase system in integration and regulation of energy fluxes between different cellular compartments.

3.1. Further problems

However, in addition to these achievements in explaining different aspects of functioning of creatine kinases, these new developments present us with new and even greater problems to explain. One of these concerns the question: for what reason is the apparent K_m for ADP in regulation of oxidative phosphorylation in vivo so high in several types of cells including heart, red muscle and liver? This high apparent K_m is characteristic for exogenous ADP, and maybe shows that there might be intracellular systems for metabolic feedback signal transduction by a mechanism of vectorial ligand conduction, proposed by Mitchell [53], due to organized cytoplasmic structures in these cells, including binding of both creatine kinase and glycolytic enzymes to the cytoskeleton ([28,55]; for reviews see Refs. [23,29]). Perhaps these organised systems transfer internal ADP very effectively, thus almost excluding the use of exogenous ADP and thus resulting in high apparent K_m values for this substrate, if it is exogenous. Or, is the outer membrane closed for the adenine nucleotides which are actively turning over inside the mitochondria and related to cytoplasmic systems via phosphocreatine and creatine? What is the structure that controls

the outer membrane? Is it connected, and if so how, to the cytoskeleton, as has been proposed [29]? How is control of respiration by creatine kinase modified by other possible regulatory systems, let us say, calcium? A very important question is the interaction between creatine kinase and adenylate kinase systems in the cells and possible 'takeover' of metabolic energy transfer and control function by the latter if the creatine kinase system is altered in pathological states or in experiments with transgenic animals [40,54]. More generally, it is important to understand the direct functional interaction between various kinases channelling energy between mitochondria and different metabolic pathways (hexokinase, creatine kinase, adenylate kinase, nucleoside diphosphokinase) [55]. Furthermore, what kind of changes in the creatine kinase MM isoenzyme molecule make it able to bind to myofibrils and build up the functionally coupled system during perinatal maturation? etc. All these questions have an even bigger impact on cellular physiology than that of the role of creatine kinase systems, since they concern the general questions of structural organization of cell energy metabolism and its importance for regulation of metabolic fluxes – all these problems may be referred to as ***structural bioenergetics*** of the cell. Thus, instead of taking the question of the physiological role of creatine kinase in the cell as being resolved, we have much more work to do and many new data to consider.

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Appendix A

1. Aerobic processes of phosphocreatine synthesis in mitochondria, based on functional coupling between creatine kinase and adenine nucleotide translocase, can be quantitatively described by a mathematical model which gives a good fit with experimental data [46–48]. This model includes:

1. The kinetic equation of the creatine kinase reaction based on its mechanism (quasi-equilibrium random binding Bi-Bi type) for description of interaction of the enzyme with its substrates in the surrounding medium;
2. A system of equations based on the probablity approach, for a description of the interaction of enzyme with mitochondrial ATP by direct transfer of ATP from translocase to creatine kinase and ADP back from creatine kinase to translocase (functional coupling) resulting in the synthesis of phosphocreatine in mitochondria and increased turnover of adenine nucleotides in mitochondria.

These equations and their use have been described in previous publications [46–48]. In the present work they were used to calculate the flux control coefficient for coupled creatine kinase.

2. In metabolic control analysis (see Ref. [41] for a detailed description), regulation of the steady-state rate of the overall reaction, or flux, J, in a metabolic system consisting of many consecutive reactions is considered to result from regulatory action at each step (reaction) of the process. Participation of each step in flux regulation is quantitatively expressed by the flux control coefficient calculated as a relative change of flux, $\mathrm{d}J/J$, resulting from the relative change of the enzyme activity, e, at that step, $\mathrm{d}e/e$, when all other parameters of the system are not changed. Thus, the flux control coefficient, C, is [41]:

$$C = (\mathrm{d}\ln J)/(\mathrm{d}\ln e)$$

It has been shown by Kacser and Burns [56] and Heinrich and Rappaport [57] that, in a homogeneous system of enzymatic reactions, the sum of flux control coefficients of all enzymes in a flux J pathway must always equal 1 (the flux control summation theorem).

The values of the flux control coefficient for mitochondrial coupled creatine kinase reaction in regulation of the steady-state rate of oxidative phosphorylation (see Fig. 2) were calculated in the following way.

For each ATP concentration, the steady-state rates of coupled reactions of oxidative phosphorylation-phosphocreatine production (V_{cp}) were calculated. The calculation were then repeated for a system with partially inhibited creatine kinase. Noncompetitive inhibition was achieved by multiplying the probabilities of formation of all intrinsic creatine kinase forms (E, E · Cr, E · ATP and E · Cr · ATP; see Ref. [46]) by the coefficient of residual activity which is O.99 for 1% inhibition, 0.98 for 2% inhibition, etc. The intrinsic forms are those formed by interaction of the enzyme with substrates in the medium. This procedure mimics the inhibition-caused decrease in the population of CK forms available for subsequent activation in the coupled reaction. Thereafter the model performs the calculations for creatine kinase activities in the coupled reactions. In these calculations the participants are the full population of translocase molecules and decreased (from given inhibition) population of creatine kinase molecules. Finally, the model calculates the flux control coefficient for each ATP concentration as:

$$C = (\mathrm{d}V_{cp}/V_{cp})/(\mathrm{d}E_{ck}/E_{ck})$$

where $\mathrm{d}E_{ck}/E_{ck}$ is the relative change of creatine kinase activity, and $\mathrm{d}V_{cp}/V_{cp}$ $(=\mathrm{d}J/J)$ is the corresponding change in the steady-state rate of coupled reactions of oxidative phosphorylation-phosphocreatine production.

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