Biochimica et Biophysica Acta, 1100 (1992) 27–32

BBABIO 43588

The role of the mitochondrial creatine kinase system for myocardial function during ischemia and reperfusion

Sibylle Soboll [a], Annette Conrad [a], Michael Keller [a] and Siegbert Hebisch [b]

[a] *Institut für Physiologische Chemie I, Universität Düsseldorf, Düsseldorf (Germany) and* [b] *Institut für Pharmakologie, Bayer AG, Wuppertal (Germany)*

(Received 10 September 1991)

Key words: Creatine kinase; Mitochondrion; Myocard; Ischemia; Reperfusion; Oxidative phosphorylation

The subcellular distribution of ATP, ADP, creatine phosphate and creatine was studied in normoxic control, isoprenaline-stimulated and potassium-arrested guinea-pig hearts as well as during ischemia and after reperfusion. The mitochondrial creatine phosphate/creatine ratio was closely correlated to the oxidative activity of the hearts. This was interpreted as an indication of a close coupling of mitochondrial creatine kinase to oxidative phosphorylation. To further investigate the functional coupling of mitochondrial creatine kinase to oxidative phosphorylation, rat or guinea-pig heart mitochondria were isolated and the mass action ratio of creatine kinase determined at active or inhibited oxidative phosphorylation or in the presence of high phosphate, conditions which are known to change the functional state of the mitochondrial enzyme. At active oxidative phosphorylation the mass action ratio was one-third of the equilibrium value whereas at inhibited oxidative phosphorylation (N_2, oligomycin, carboxyatractyloside) or in the presence of high phosphate, the mass action ratio reached equilibrium values. These findings show that oxidative phosphorylation is essential for the regulation of the functional state of mitochondrial creatine kinase. The functional coupling of the mitochondrial creatine kinase and oxidative phosphorylation indicated from the correlation of mitochondrial creatine phosphate/creatine ratios with the oxidative activity of the heart in situ as well as from the deviation of the mass action ratio of the mitochondrial enzyme from creatine kinase equilibrium at active oxidative phosphorylation in isolated mitochondria is in accordance with the proposed operation of a creatine shuttle in heart tissue.

Introduction

The mitochondrial and extramitochondrial creatine systems are the constituents of the 'creatine shuttle' (for a recent review see [1]) in heart and skeletal muscle. The creatine kinase shuttle not only channels mitochondrial ATP generated by oxidative phosphorylation to the myofibrils for muscle contraction but rises the cytosolic phosphorylation potential above the level measured in non excitable tissues, e.g., the liver [2], and therefore enhances energy yield of ATP hydrolysis for muscle work.

The presumption for effective channelling of mitochondrial ATP towards creatine phosphate (CP) formation at the mitochondrial site is a functional coupling of mitochondrial creatine kinase (CK) located at the outside of the inner mitochondrial membrane to mitochondrial ATP production. Several groups have demonstrated that mitochondrial CK is functionally coupled to mitochondrial adenine nucleotide translocase [3–5] or oxidative phosphorylation [6–8] whereas others postulate compartmentation of adenine nucleotides between mitochondria, intermembrane and cytosolic space [9]. The group of Wallimann [10] showed that it is an octameric form of mitochochondrial CK which associates with the inner mitochondrial membrane whereas a dimer is prevalent in solution. As a consequence of the functional coupling of mitochondrial CK with oxidative phosphorylation, the mass action ratio of mitochondrial CK should be shifted away from thermodynamic equilibrium towards CP synthesis [4].

Only few experiments have related the status of mitochondrial CK to different functional states in the intact heart, like Bittl et al. [11], who correlated the associated mitochondrial CK with heart function in mitochondria isolated from normoxic, ischemic and reoxygenated hearts.

In a previous study with rat gastrocnemius muscle we demonstrated an increase in mitochondrial CP and a decrease in mitochondrial creatine (Cr) with increasing isotonic stimulation [12], while inverse changes

Correspondence: S. Soboll, Institut für Physiologische Chemie I, Universität Düsseldorf, Moorenstrasse 5, 4000 Düsseldorf, Germany.

occurred in cytosolic CP and Cr. This indicates a high rate of CP synthesis by mitochondrial CK operating under conditions of high respiratory activity.

In this study we wanted to know whether the mitochondrial/cytosolic distribution of the creatine kinase reactants in the intact heart reflects a higher activity of the creatine shuttle under conditions of high oxidative activity, e.g., during adrenergic stimulation or during reperfusion. For this purpose isolated perfused guinea-pig hearts in different functional states were freeze-clamped, freeze-dried and the mitochondrial and extramitochondrial concentrations of ATP, ADP, CP and Cr determined after fractionation of the tissue in non-aqueous solvents [13].

Furthermore, the mass action ratio of the CK reaction as a parameter for the functional state of the mitochondrial enzyme was determined in the supernatant of suspensions of isolated mitochondria during hypoxia, reoxygenation and under high-phosphate concentrations.

Methods

Isolated heart perfusion

Guinea-pigs of either sex with a body weight of 200–300 g, fed with a standard diet (Altromin, Lage, FRG) and water ad libitum, were killed by a blow on the head, the thorax was opened, the pulmonal artery incised and the aorta cannulated. Thereafter, the heart was disconnected from the venae cavae and the distal aorta was transferred with the lungs to the perfusion apparatus. There the heart was perfused retrogradely (constant flow 10 ml/min) with carbogen (95% O_2/5% CO_2)- gassed Krebs–Henseleit bicarbonate buffer supplied with 10 mM glucose and a final $CaCl_2$ concentration of 1.8 mM [14].

The left ventricular pressure was measured by inserting a Latex-balloon (HSE, March, Germany) into the left ventricle via the left atrium and mitral valve. The balloon was filled with saline up to a preload of 5 mmHg and was connected to a pressure transducer (Bell and Howell 4-327-I, Pasadena, CA, USA). The signal was recorded continuously on a strip chart recorder (Gould Recorder 2800, Gould, Cleveland, OH, USA). The coronary perfusion pressure was determined by a second pressure transducer between the roller pump and the aortic cannula. Its signal was evenly recorded. The heart rate was taken from the left ventricular pressure curve. In some of the experiments the p_{O_2} in the arterial and venous perfusate was measured polarographically with Clarke type electrodes (Type 2000, Eschweiler, Kiel, Germany). In these experiments the pulmonal artery was cannulated and the flow was measured by means of a flow-meter (HSE, March, Germany). All hearts were paced via a graphite electrode placed on the right atrium (square pulses, 2 ms duration, 10 V amplitude, 4 Hz frequency).

All experiments were started after an equilibration period of 1 h. KCl, atractyloside and isoprenaline were infused into the aortic cannula with a flow of 0.1 ml/min, the concentrations given in the results section are the effluent perfusate concentrations. All measurements as well as the freeze-clamp were made under steady-state conditions.

Determination of mitochondrial and extramitochondrial metabolite concentrations

Fractionation of heart tissue in non-aqueous solvents [13]. From the freeze-clamped guinea-pig hearts, the left ventricular tissue was isolated under liquid nitrogen, ground, and lyophilised at −40°C. The tissue was sonicated in heptane/CCl_4, density 1.23 g/ml, in 5-s intervals for 5 min under continuous cooling in heptane/solid CO_2; the homogenate was fractionated by density gradient centrifugation in heptane/CCl_4, the densities ranging from 1.29–1.38 g/ml. Centrifugation (16500 × *g* for 3 h) yielded 8 fractions each containing different proportions of mitochondrial and cytosolic protein. In each fraction the specific activities of marker enzymes for cytosol, phosphoglycerate kinase and mitochondria, citrate synthase, respectively, as well as the contents of ATP, ADP, CP and Cr were determined by enzymatic analysis [15] and mitochondrial and extramitochondrial contents were obtained by extrapolation from the contents in the fractions to pure mitochondrial and extramitochondrial fractions. The contents were converted into concentrations using 1.0 µl/mg mitochondrial protein and 3.5 µl/mg extramitochondrial protein as subcellular water spaces, respectively [16,17].

The extrapolation as well as the methodological discussion are published in Ref. [13]. The fractionation of intact tissue using non-aqueous solvents originally developed for liver has been successfully applied to isolated rat Langendorff heart [18,19] and isolated working guinea-pig heart [20]. As in our paper, mitochondrial ATP/ADP ratios in heart ranged from 2 to 4 depending on experimental conditions (similar to values found in heart mitochondria isolated in sucrose media [21]) and cytosolic ratios of free ATP/free ADP were in the range from 100–500, depending on work load or oxygen supply, demonstrating the validity of the method with regard to energy metabolism.

Isolation and incubation of heart mitochondria

Heart mitochondria from rats or guinea-pigs were isolated essentially according to Ref. 22 in a medium containing 0.25 M sucrose, 10 mM Tris, 5 mM KH_2PO_4, 20 mM KCl, 0.2 mM EDTA (pH 7.2), using 1 mg trypsin for 2 guinea-pig hearts or 3 rat hearts. Rat heart as well as guinea-pig heart mitochondria had

respiratory control ratios between 8 and 15 in the presence of 2.5 mM glutamate and 2 mM malate in the absence of magnesium. In the presence of 5 mM $MgCl_2$ the respiratory control ratios were 6–8 indicating moderate contamination with Mg2 + -ATPase similar to preparations of others [4,22]. The rate of respiration was in the range of 250 to 300 nmol O_2/min per mg of mitochondrial protein at 37°C. Phosphoglycerate kinase activity as marker for cytosolic contamination was absent from the mitochondrial fraction. As tested in some representative experiments, results regarding the mass action ratio of creatine kinase were the same with guinea-pig and rat heart mitochondria.

About 1 mg mitochondrial protein/ml was incubated in the same medium as used for isolation in the presence of 5 mM $MgCl_2$, 1 mM ATP and 10 mM Cr for 20 min under continuous stirring. Hypoxic incubations were performed in a closed reaction chamber under continuous nitrogen gassing. Oxygen concentration under this condition was maximal 1%. Reoxygenation with 21% oxygen was performed after 20 min of hypoxia for 20 min in an open Erlenmeyer flask. For each sample 100 μl of the suspension was centrifuged and the supernatant deproteinised in perchloric acid and neutralised. ATP, ADP, CP and Cr was determined in the mitochondria free supernatant using enzymatic analysis [15] and the mass action ratio K_{app} of creatine kinase calculated according to Lawson and Veech [23].

$$K_{app} = [ATP][Cr]/[ADP][CP].$$

The equilibrium constant was determined in the same medium at the same pH using 2.5 units of soluble bovine creatine kinase/ml (Böhringer, Mannheim, Germany) essentially as described above from the reactants of the creatine kinase to be 122 ± 17.

For incubation at different phosphate concentrations guinea-pig heart mitochondria were used. They were incubated in the presence of 1 to 20 mM potassium phosphate in the isolation medium for 20 min and then stimulated with 5 mM $MgCl_2$, 1 mM ATP and 10 mM Cr for 5 min. Determination of the mass action ratio of CK was determined as above.

Materials

All enzymes and coenzymes were either from Böhringer or from Sigma Chemie (München, Germany), chemicals were from Merck (Darmstadt, Germany) and all were of highest purity, available.

Results and Discussion

Isolated heart perfusion

The following metabolic situations were induced in Langendorff hearts for fractionation studies (Table I): 1. Control hearts supplied with 10 mM glucose. 2. 40 mM KCl + 40 μM atractyloside; arrested hearts which use energy only for basal metabolism. The creatine shuttle cannot operate due to lack of mitochondrial ATP, since adenine nucleotide transport is inhibited. This is reflected in a very low oxygen consumption. 3. Isoprenaline 5 μg/l; adrenergic stimulation increases heart work (LVPP × frequency (+ 111%)), lactate output more than 2-fold and respiration by 30%. A high activity of the "creatine shuttle" is to be expected. 4. 30 min of ischemia; lack of oxygen supply leads to a dramatic decrease in oxidative phosphorylation (respiration) and work; the "creatine shuttle" is not operating at inhibited oxidative phosphorylation. 5. 30 min ischemia followed by 30 min of reperfusion; after 30 min of reperfusion, left ventricular function and oxygen consumption have been returned to control values; transient increased oxidative activity with high turnover of the creatine shuttle.

Changes in the subcellular adenine nucleotide and creatine systems in the heart in situ (Tables II and III)

In control hearts the mitochondrial ATP level as well as the ATP/ADP ratio is the lowest from all

TABLE I

Functional parameters and metabolic rates in isolated perfused guinea-pig hearts in different metabolic states

n = 7–14 ± S.E.; n for oxygen consumption measurements in brackets. For a description of metabolic situations see Results.

	PP (mmHg)	HR (min^{-1})	LVPP × HR (mmHg/min)	Oxygen (μmol/min)	Lactate (μmol/min)
Control	63 ± 10	183 ± 17	13302 ± 2350	5.5 ± 0.5	0.09 ± 0.09 (8)
KCl + atractyloside	103 ± 20	-	-	1.6–2.9	0.14 ± 0.07 (2)
Isoprenaline	56 ± 23	276 ± 19	29100 ± 6400	7.0–7.3	0.69 ± 0.11 (2)
Reperfusion	77 ± 23	167 ± 15	12200 ± 2150	5.1 ± 0.7	0.20 ± 0.15 (4)

TABLE II

Mitochondrial concentrations (mmol · l) of ATP, ADP, CP and Cr in isolated perfused guinea pig hearts

$n = 7-14 \pm$ S.E.

	ATP	ADP	CP	Cr	ATP/ADP	CP/Cr
Control 10 mM glucose + 40 mM KCl;	16.5 ± 2.9	8.8 ± 1.4	2.5 ± 1.3	27.4 ± 3.8	2.4 ± 0.6	0.13 ± 0.04
40 μM atractyloside	32.0 ± 3.4	8.7 ± 1.3	0.4 ± 2.3	24.5 ± 3.4	4.6 ± 0.9	0.04 ± 0.02
+ isoprenaline (5 μg/l)	21.2 ± 2.6	5.7 ± 1.3	7.8 ± 1.1	19.3 ± 3.4	4.9 ± 0.5	0.51 ± 0.13
Ischemia 30 min	18.8 ± 2.4	6.0 ± 0.8	0.9 ± 0.7	35.1 ± 3.8	3.4 ± 0.3	0.04 ± 0.02
Reperfusion 30 min	25.0 ± 4.1	6.4 ± 1.4	15.0 ± 2.9	39.0 ± 6.5	4.9 ± 1.0	0.48 ± 0.10

conditions examined, whereas the corresponding cytosolic values are the highest. This is consistent with a high energisation of the mitochondrial membrane, since the distribution of ATP and ADP should reflect the mitochondrial membrane potential, i.e., the difference between cytosolic and mitochondrial ATP/ADP ratios should increase as the membrane potential increases. With atractyloside the increase in the mitochondrial ratio and the decrease in the cytosolic ratio reflect the inhibition of mitochondrial adenine nucleotide translocase. Similar changes are observed during ischemia ± reperfusion; it has been shown that during hypoxia fatty acid metabolites are considerably increased compared to the control [19]. Since it is known that long chain acyl CoA inhibit mitochondrial adenine nucleotide translocase [24] it is feasible that the changes in adenine nucleotides also reflect an inhbition of adenine nucleotide transport; as suggested in an earlier work [19] this would prevent a degradation of cytosolic ATP by mitochondrial ATPase under conditions of low mitochondrial energy supply. Whereas, however, in that previous study a decrease in the mitochondrial ATP/ADP ratio was observed after 90 min of ischemia, the present study with only 30 min showed an increase, as would be expected when only adenine nucleotide translocation is inhibited.

With isoprenaline the mitochondrial ATP/ADP ratio is increased whereas the cytosolic ratio is not significantly changed compared to the control despite the dramatic increase in cytosolic energy turnover (Table I). The higher mitochondrial ATP/ADP ratio under this condition probably reflects a higher rate of mitochondrial ATP synthesis leading to a new steady state of the mitochondrial ATP system

The free cytosolic ATP/ADP ratios calculated from the cytosolic CP/Cr ratios do not correspond to the total cytosolic ratios but rather to the actual work output and the energy supply of the heart. Thus, it is highest in KCl-arrested hearts with very low work output; despite the inhibition of mitochondrial ATP transport, the ATP synthesis via glycolysis is sufficient so that the cytosolic creatine kinases poise the free ATP/ADP ratio at a very high level. With adrenergic stimulation the cytosolic free ATP/ADP ratio is not decreased compared to the control although work output is more than doubled, since also ATP synthesis via oxidative phosphorylation and glycolysis are stimulated.

The free cytosolic ATP/ADP ratio is lowest in ischemia where oxidative phosphorylation is inhibited by lack of oxygen supply and also ATP synthesis by glycolysis is inhibited because lactate accumulates fast at zero flow. It returns close to control values when flow and oxygenation are reestablished.

On the other hand, the mitochondrial creatine system closely reflects the oxidative activity of the hearts: CP as well as the CP/Cr ratio are enhanced with isoprenaline and after 30 min of reperfusion, situations with high transient oxidative activity, whereas they are lowest with atractyloside and during ischemia. The rise

TABLE III

Extramitochondrial concentrations (mmol · l) of ATP, ADP, CP and Cr in isolated perfused guinea-pig hearts

$n = 7-14 \pm$ S.E.; free cytosolic ATP/ADP ratios were calculated from the cytosolic creatine kinase reactants [23].

	ATP	ADP	CP	Cr	ATP/ADP		CP/Cr
					total	free	
Control, 10 mM glucose + 40 mM KCl;	8.2 ± 0.9	1.2 ± 0.1	11.9 ± 1.2	6.4 ± 0.9	8.4 ± 1.8	303 ± 31	2.0 ± 0.2
40 μM atractyloside	6.3 ± 0.9	2.1 ± 0.2	17.8 ± 1.4	7.8 ± 1.1	3.4 ± 0.5	463 ± 90	3.0 ± 0.6
+ isoprenaline (5 μg/l)	9.3 ± 0.8	1.6 ± 0.1	14.2 ± 1.1	7.4 ± 0.8	6.8 ± 1.6	305 ± 28	2.1 ± 0.2
Ischemia 30 min	6.5 ± 0.8	2.1 ± 0.2	4.0 ± 0.5	12.8 ± 2.1	3.1 ± 0.2	52 ± 6	0.4 ± 0.04
Reperfusion 30 min	5.0 ± 0.4	1.6 ± 0.2	8.8 ± 1.3	4.8 ± 0.7	3.5 ± 0.4	278 ± 30	1.9 ± 0.2

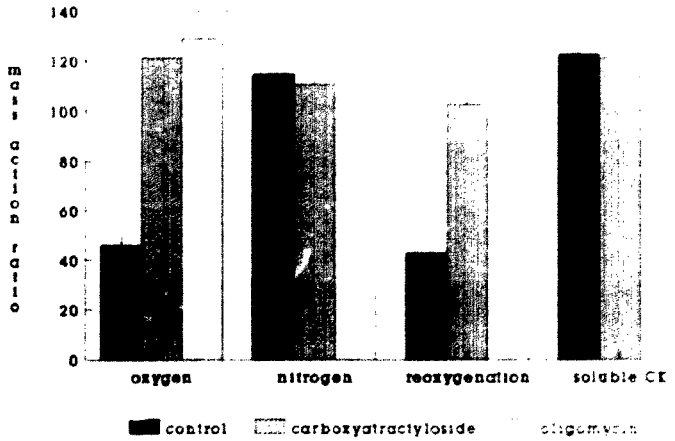

Fig. 1. Dependence of the mass action ratio of mitochondrial creatine kinase from coupling to oxidative phosphorylation. Isolated rat heart mitochondria, incubation conditions see Methods. $n = 4 \pm$ S.E. $K_{eq} = 122$ at pH 7.2.

in mitochondrial creatine during ischemia ± reperfusion can be attributed to an increase in the permeability of mitochondria for creatine during phases of oxygen deficiency [25]. The high mitochondrial CP on reperfusion, on the other hand, reflects a higher rate of CP synthesis by mitochondrial CK coupled to oxidative phosphorylation [6–8,11] and not a permeability change due to transient hypoxia: This becomes apparent from the low mitochondrial CP contents in control and arrested hearts (Table II). Further, the increase in the mitochondrial CP/Cr closely corresponds to oxidative activity of the heart in this study as well as in earlier reports [12,20] and is not related to damaging conditions for the heart. However, it is not clear how CP reaches the mitochondrial matrix, since no transport system for CP is known in the mitochondrial membrane. As suggested in an earlier report [12], it is feasible that CP synthesised by the mitochondrial CK at high rates is partially transported by the adenine nucleotide translocase into the matrix due to the close coupling of mitochondrial creatine kinase with the translocase, since this carrier also accepts other molecules with energy-rich phosphate bonds like phospho*enol*pyruvate [26].

Taken together, the finding of CP in the mitochondria and the close correlation between the mitochondrial CP/Cr ratio and the oxidative activity not only in heart muscle as shown here but also in skeletal muscle [12], are consistent with the creatine shuttle operating in these tissues and with the suggestion that mitochondrial creatine kinase is closely coupled to oxidative phosphorylation.

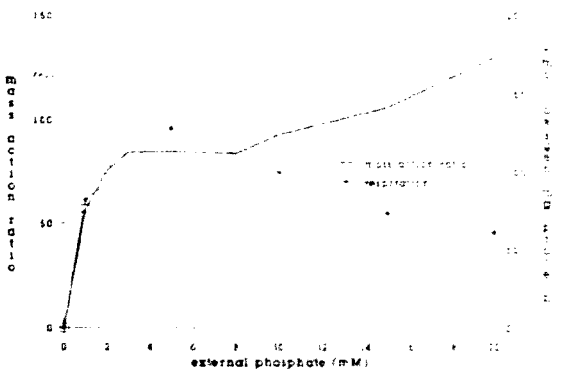

Fig. 2. Dependence of the mass action ratio of creatine kinase and of respiration in guinea-pig heart mitochondria from external phosphate. Incubation conditions see methods. $n = 10–15 \pm$ S.E. for mass action ratios and $4 \pm$ S.E. for respiration rates. $K_{eq} = 122$ at pH 7.2.

Mass action ratio of mitochondrial creatine kinase in different functional states in isolated heart mitochondria (Figs. 1 and 2)

In order to confirm this hypothesis, the mass action ratio of mitochondrial CK was measured in isolated heart mitochondria under conditions of hypoxia and reoxygenation which should simulate ischemia and reperfusion, states of physiological relevance in the heart. The functional role of the creatine shuttle implies that the mitochondrial CK operates in the opposite direction as the extramitochondrial systems, i.e., in the direction of CP synthesis. The thermodynamic equilibrium of this reaction, however, favors ATP synthesis [23] and it is generally believed that the extramitochondrial CK establish equilibrium even during increased muscle work [27]. We compared the mass action ratio of CK established from isolated mitochondria with the equilibrium ratio. A displacement of the mass action ratio K_{app} from the equilibrium ratio

indicates that an enzyme is by some reason not in free equilibrium with its reactants. Regarding mitochondrial CK, the cause is suggested to be oxidative phosphorylation closely located by this enzyme, creating a microenvironment with concentrations of the reactants different from those measured in the extramitochondrial medium [4,9], i.e., high ATP and very low ADP concentrations. As shown in Fig. 3 the mass action ratio of mitochondrial CK is only one third of the equilibrium ratio in normoxic rabbit heart mitochondria similar to the experiments in [4]. On the other hand, the mass action ratio of CK is close to the equilibrium value when oxidative phosphorylation is inhibited, e.g., in hypoxic mitochondria, with carboxyatractyloside, an inhibitor of mitochondrial adenine nucleotide translocase, or with oligomycin, an inhibitor of mitochondrial ATP synthase. Both inhibitors have no influence on soluble CK (Fig. 1). Therefore we conclude that coupling of mitochondrial CK to oxidative phosphorylation followed by net CP synthesis, expressing thermodynamic restrictions, is dependent on rates of ATP synthesis and ATP/ADP transport across the mitochondrial membrane.

In the heart, during hypoxia cellular phosphate is increased (cytosolic phosphate rises from 9.3 mM ± 0.3 in control hearts to 15 mM ± 1.7 in ischemic hearts, respectively). High phosphate is able to dissociate mitochondrial CK from the inner mitochondrial membrane [28]. Consequently under high phosphate the mitochondrial CK is expected to behave like a soluble CK and establish thermodynamic equilibrium. This is confirmed in Fig. 2: mitochondria were incubated with increasing phosphate concentrations and the mass action ratio determined from the ATP, ADP, CP and Cr concentrations in the supernatant. Whereas, at low concentrations of phosphate (below 5 mM) mitochondrial CK is inhibited due to the inhibition of oxidative phosphorylation (lower trace, Fig. 2A, at a maximum of oxidative phosphorylation (at about 5 mM P_i) the mass action ratio is still significantly lower than the equilibrium value which is reached at phosphate concentrations above 15 mM when CK is dissociated from the mitochondrial membrane.

Summarizing, the measured mass action ratios of mitochondrial CK in isolated mitochondria under hypoxic and normoxic conditions confirm our hypothesis that during high rates of oxidative phosphorylation mitochondrial CK is not able to establish thermodynamic equilibrium due to its functional coupling to oxidative phosphorylation. This close coupling leads to a netto synthesis of CP at the mitochondrial site, which is the basis for the operation of the creatine shuttle.

Acknowledgement

We thank Sonja Gründel, Brigida Ziegler and Götz Künkeler for expert technical assistance. This work was supported by Bayer Co/Leverkusen.

References

1 Bessman, S.P. and Carpenter, C.L. (1985) Annu. Rev. Biochem. 54, 831–862.
2 Soboll, S., Scholz, R. and Heldt, H.W. (1978) Eur. J. Biochem. 87, 377–390.
3 Moreadith, R.W. and Jacobus, W.E. (1982) J. Biol. Chem. 257, 899–905.
4 Saks, V.A., Kuznetsov, A.V., Kupriyanov, V.V., Miceli, M.V. and Jacobus, W. (1985) J. Biol. Chem. 260, 7757–7764.
5 Barbour, R.L., Ribaudo, J. and Chan, S.H.P. (1984) J. Biol. Chem. 259, 8246–8251.
6 Biermans, W., Bakker, A. and Jacob, W. (1980) Biochim. Biophys. Acta 1018, 225–228.
7 Borrebaek, B. (1980) Arch. Biochem. Biophys. 203, 827–829.
8 Kuznetsov, A.V., Kuchua, Z.A., Vassileva, E.V., Medvedeva, E.N. and Saks, V.A. (1989) Arch. Biochem. Biophys. 269, 176–190.
9 Gellerich, F.N., Schlame, M., Bohnensack, R. and Kunz, W. (1987) Biochim. Biophys. Acta 890, 117–125.
10 Marcillat, O., Goldschmidt, D., Eichenberger, D. and Vial, C. (1987) Biochim. Biophys. Acta 890, 233–241.
11 Bittl, J.A., Wersfeld, M.L. and Jacobus, W.E. (1986) J. Biol. Chem. 260, 208–214.
12 Hebisch, S., Sies, H. and Soboll, S. (1986) Pflüger's Arch. 406, 20–24.
13 Soboll, S., Elbers, R. and Heldt, H.W. (1979) Methods Enzymol. 56, 201–206.
14 Opie, L.H. (1965) J. Physiol. London 180, 529–541.
15 Bergmeyer, H.U. (1984) Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Vol. III, Verlag Chemie, Weinheim.
16 Illingworth, J.A., Ford, W.C.L., Kobayashi, K. and Williamson, J.R. (1975) in Recent Advances on Cardiac Structure and Metabolism (Roy, R.E. and Harris, P., eds.), pp. 271–290, University Park Press, Baltimore.
17 Morgan, H.E., Henderson, M.J., Regen, D.M. and Park, C.R. (1961) J. Biol. Chem. 236, 253–261.
18 Kauppinen, R.A., Hiltunen, K. and Hassinen, I. (1980) FEBS Lett. 112, 273–276.
19 Hütter, J.F., Alves, C. and Soboll, S. (1990) Biochim. Biophys. Acta 1016, 244–252.
20 Bünger, R. and Soboll, S. (1981) Hoppe-Seyler's Z. Physiol. Chem. 362, 125–132.
21 Davis, E.J. and Lumeng, L. (1975) J. Biol. Chem. 250, 2275–2282.
22 Jacobus, W. and Saks, V.A. (1982) Arch. Biochem. Biophys. 219, 167–178.
23 Lawson, R.J. and Veech, R.L. (1979) J. Biol. Chem. 254, 6528–6537.
24 Pande, S.V., Goswami, T. and Parvin, R. (1984) Am. J. Physiol. 247, 25–34.
25 Altschuld, R.A., Nerola, A.J. and Brierley, G.P. (1975) J. Mol. Cell. Cardiol. 7, 451–462.
26 Brocks, D.G., Siess, E.A. and Wieland, O.H. (1980) Eur. J. Biochem. 113, 29–45.
27 Shoubridge, E.A., Briggs, R.W. and Radda, G.K. (1982) FEBS Lett. 140, 288–292.
28 Hall, N. and Deluca, M. (1980) Arch. Biochem. Biophys. 201, 674–677.