Modulation of Mitochondrial Contact Sites Formation in Immature Rat Heart

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Abstract. Creatine phosphokinase-mediated transport of energy from the site of production to the site of consumption is a key process for meeting the energy-demands of reactions in cytosol. The mitochondrial creatine phosphokinase (mCPK) plays an important role in this process, with the enzyme activity localized particularly in the mitochondrial contact sites (MiCS) Earlier studies in adult animals have shown that the formation of MiCS varies in response to the energy demand and the physiological state of the heart, and it is stimulated by an increase in $[Ca^{2+}]_i$ However, there is little known about MiCS formation in juvenile hearts, characterized by metabolism different from adult hearts. In the present study we investigated the modulation of MiCS formation via Ca^{2+} in hearts of 14-day-old rats. The moderate response of MiCS to various stimuli (elevated extracellular Ca^{2+}, diltiazem, cardiac arrest by Cd^{2+}) may refer to a still increased intracellular Ca^{2+} concentration, the incomplete development of mitochondrial energy production as well as to persistingly high energy demand of the developing heart

Key words: Immature heart – Mitochondria – Creatine kinase – Mitochondrial contact sites

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Introduction

Creatine phosphokinase (CPK) plays an important role in the intracellular energy transport, particularly in tissues with high energy demand, such as heart, skeletal muscle, brain etc. (Wallimann et al. 1986). The enzyme exhibits specific isoforms, localized in the cytoplasm, on myofibrils and in the mitochondria. Among them, a special function is fulfilled by the mitochondrial isoenzyme that, in dimeric form, is present in the space between the inner and outer membranes of the mitochondria. However, it can be organized also in octameres that were found to be localized in the mitochondrial contact sites (MiCS). MiCS are loci where the inner and outer mitochondrial membranes become connected by the structure consisting of a molecule of porin in the outer membrane, the octameric mitochondrial creatine phosphokinase (mCPK) in the inter-membrane space, and the molecule of ATP/ADP translocase in the inner mitochondrial membrane. MiCS are believed to participate in the transfer of energy through the mitochondrial membranes (Brdiczka 1991, Wyss et al. 1992). Biermans et al. (1989) utilized the presence of mCPK octameres in MiCS for cytochemical detection of these structures. The latter study also revealed that the amount of MiCS present in cardiac mitochondria increases in parallel with the increase in metabolic activity of the heart. Our previous studies revealed that in adult hearts an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) may act as a signal for both, an increase in the metabolic activity of the heart as well as an enhanced formation of the MiCS (Bakker et al. 1993, 1994, Ziegelhoffer-Mihalovičová et al. 1997). It has been well documented that the metabolism of rat hearts undergo most dramatic changes during first 2 weeks of postnatal development (Wittmich 1997). There is a shift from anaerobic metabolism in the prenatal period to aerobic metabolism in the adulthood (Hohl 1997, Tribulová et al. 1997). This is accompanied by changes in calcium handling. So, we were interested in the formation of MiCS and in the modulation of this process in immature rat hearts.

Materials and Methods

Hearts of 36 14-day-old Wistar rats, divided into 6 groups (6 animals each), were excised and Langendorff-perfused at 37°C for 15 min stabilisation period (SP) with Krebs-Henseleit solution (K-H) containing (in mmol l^{-1}) 118.5 NaCl, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 1.18 KCl, 1.2 $MgSO_4$, 11.5 glucose, and 1.6 $CaCl_2$. It was gassed with a mixture of 95% oxygen and 5% carbon dioxide. Subsequently, the hearts were perfused for additional 15 min as follows: **Group 1** (Controls) – the same K-H solution as above, **Group 2** (High Ca^{2+}) – K-H containing 2.2 mmol l^{-1} Ca^{2+}, **Group 3** (Controls with diltiazem) – K-H containing 1.6 mmol l^{-1} Ca^{2+} + 5 μmol l^{-1} diltiazem, **Group 4** (High Ca^{2+} with diltiazem) – K-H containing 2.2 mmol l^{-1} Ca^{2+} + 5 mmol l^{-1} diltiazem, **Group 5** (Ca-paradox-like model) 3

min Ca^{2+}-depletion and 12 min Ca^{2+}-repletion, **Group 6** (Cardiac arrest) – after SP administration of 5 mmol l^{-1} $CdCl_2$

After completion of the experiment, the hearts were perfusion-fixed and further processed for cytochemical determination of octameric mCPK according to the method of Biermans et al (1989), (for details see Ziegelhoffer-Mihalovičová et al 1997) The method is based on reduction of a thiocarbamyl nitro blue tetrazolium chloride salt in the presence of lactate and glucose-6-phosphate dehydrogenases Thin sections of embedded tissue slices were examined in electron microscope Stereological method (Baddeley et al 1986) was used to evaluate the MiCS surface to mitochondria surface ratio (S_S) The testing grid was applied over the electronmicrographs and the ratio of intersections of cycloids with MiCS and intersections of cycloids with mitochondrial membranes was counted

Results

The baseline values of rate-pressure product, parameter of cardiac performance (heart rate×developed pressure, RPP), of the left ventricle after SP did not differ between the groups Perfusion with high Ca^{2+} concentration (group 2) increased the RPP by 42 6% as compared to control values (group 1) Diltiazem (group 3) decreased RPP by 71% while in the presence of 2 2 mmol l^{-1} Ca^{2+}, it was

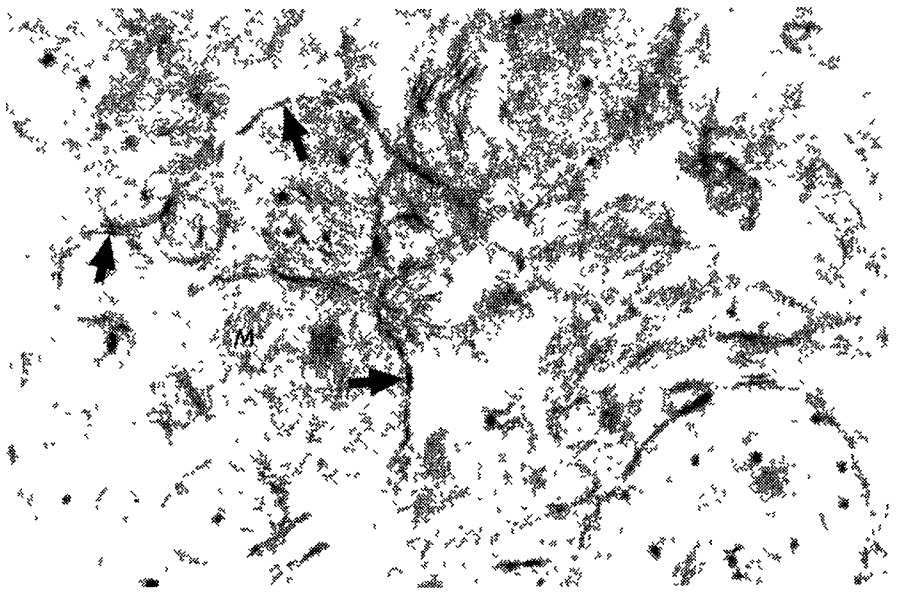

Figure 1. Cytochemically detected MiCS in heart mitochondria of 14-days old rats control group *arrows* mitochondrial contact sites, *M* – mitochondria

decreased by 46.9% only. Calcium-paradox-like intervention (group 5) decreased heart performance by 74.8%.

The electronmicrograph (Fig. 1) shows an example of the histochemically detected MiCS in control rat heart.

Results from the stereological evaluation of MiCS frequency are shown in Fig. 2 as the MiCS surface to mitochondria surface ratio (S_S).

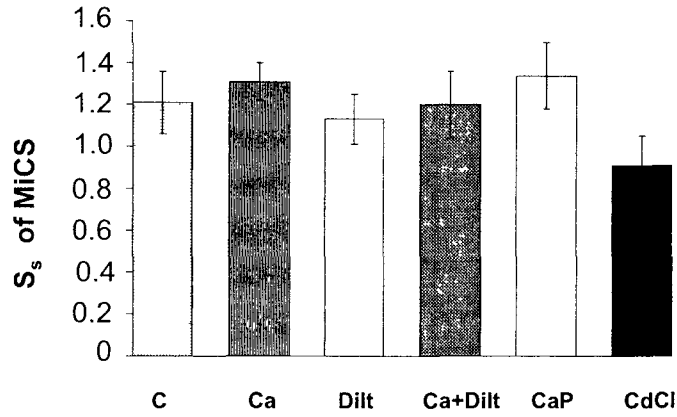

Figure 2. MiCS surface to mitochondria surface ratio (S_S) in hearts of 14-days old rats. *C* = controls, *Ca* = high calcium, *Dilt* = controls with diltiazem, *Ca+Dilt* = high calcium with diltiazem, *CaP* = Ca-paradox-like model, *CdCl* = cardiac arrest, *error bars* = ±S E M

The detection of MiCS in the first group of hearts, serving as controls, showed very abundant staining of contact sites with the precipitate from the cytochemical detection of mCPK.

Perfusion of hearts with increased extracellular $[Ca^{2+}]_e$ (group 2) as well as the Ca^{2+} overload model (group 5) failed to induce any significant increase in the amount of MiCS. Diltiazem depressed the formation of MiCS in hearts perfused with normal $[Ca^{2+}]_e$ only nonsignificantly. Relaxed hearts of group 6 (cardiac arrest) revealed only nonsignificant decrease in the amount of MiCS.

Discussion

During perinatal and early postnatal development, hearts undergo numerous changes in their metabolism connected to the shift from anaerobic to aerobic energy production. The oxidative capacity of cardiac mitochondria increases during this period reaching its adult values at 15–20 days postnatally (Glatz and Veerkamp

1982). In the control of mitochondrial respiration in the heart, Ca^{2+} plays an important role in the activation of mitochondrial enzymes (Hansford 1994).

Our results indicate that, in 14-day old rat hearts, the response of MiCS formation to Ca^{2+}-signalling is altered as compared to adult rat hearts. This is partially due to the alterations in calcium handling and calcium tolerance in immature rat hearts (Mahony 1996). Increased intracellular Ca^{2+} transients (Vornanen 1996) seem to maintain the formation of MiCS persistingly high.

The high energy demand of the developing heart present even in control situation requires effective transport of energy from mitochondria to the cytosol, provided by MiCS. The weak response of MiCS formation to Ca^{2+} stimuli may result from the fact that, to keep the transport of energy produced in the maturing mitochondria sufficient, maximal amount of transport places rather than downregulation of MiCS is required.

Acknowledgements. Authors are indebted to Mrs. A. Brichtová, I. Bernaert and M. De Bie for their perfect technical assistance. This study was supported by VEGA Grants No. 2/4044/97 and 2/4045/97.

References

Baddeley A. J., Gundersen H. J. G., Cruz-Orive L. M. (1986): Estimation of surface area from vertical sections. J. Microsc. (Oxford) **142,** 259–276

Bakker A., De Bie M., Bernaert I., Ravingerová T., Ziegelhöffer A., Van Belle H., Jacob W. (1993): Increased extracellular calcium concentrations in the isolated rat heart: effects on mitochondrial contact site formation. Eur. J. Morphol. **31,** 46–50

Bakker A., Bernaert I., De Bie M., Ravingerová T., Ziegelhöffer A., Van Belle H., Jacob W. (1994): The effect of calcium on mitochondrial contact sites: a study on isolated rat hearts. Biochim. Biophys. Acta **1224,** 583–588

Biermans W., Bernaert I., De Bie M., Nijs B., Jacobs W. (1989): Ultrastructural localisation of creatine kinase activity in the contact sites between inner and outer mitochondrial membranes in rat myocardium. Biochim. Biophys. Acta **974,** 74–80

Brdiczka D. (1991): Contact sites between mitochondrial envelope membranes – structure and function in energy-transfer and protein-transfer. Biochim. Biophys. Acta **1071,** 291–312

Glatz J. F. C., Veerkamp J. H. (1982): Postnatal development of palmitate oxidation and mitochondrial enzyme activities in rat cardiac and skeletal muscle. Biochim. Biophys. Acta **711,** 327–335

Hansford R.G. (1994): Role of calcium in respiratory control. Med. Sci. Sports Exerc. **26,** 44–51

Hohl Ch. M. (1997): Effect of respiratory inhibition and ischemia on nucleotide metabolism in newborn swine cardiac myocytes. In: The Developing Heart (Eds. B. Ošťádal, M. Nagano, N. Takeda and N. S. Dhalla), pp. 393–405, Lippincott-Raven Publishers, Philadelphia, USA

Mahony L. (1996): Regulation of intracellular calcium concentration in the developing heart. Cardiovasc. Res. **31,** E61–E67

Tribulova N, Okruhlicova L, Ziegelhoffer-Mihalovičova B, Slezak J (1997) Myocardial ischemia tolerance differences between immature and adult rat hearts. In: The Developing Heart (Eds. B. Oštadal, M. Nagano, N. Takeda and N. S. Dhalla), pp. 407–425, Lippincott-Raven Publishers, Philadelphia, USA

Vornanen M. (1996) Excitation-contraction coupling of the developing rat heart. Mol. Cell. Biochem. **163/164**, 5–11

Wallimann T., Wegman G., Moser H., Huber R., Eppenberger H. M. (1986) High content of creatine kinase in chicken retina: compartmentalized location of creatine kinase isoenzymes in photoreceptor cells. Proc. Natl. Acad. Sci. USA **83**, 3816–3819

Wittnich C. (1997) Postnatal development: metabolic and functional determinants of response to oxygen stress. In: The Developing Heart (Eds. B. Oštadal, M. Nagano, N. Takeda and N. S. Dhalla), pp. 443–457, Lippincott-Raven Publishers, Philadelphia, USA

Wyss M., Smeitink J., Wevers R. A., Wallimann T. (1992) Mitochondrial creatine kinase: a key enzyme of aerobic energy metabolism. Biochim. Biophys. Acta **1102**, 119–166

Ziegelhoffer-Mihalovičova B., Okruhlicova L., Tribulova N., Ravingerova T., Volkovova K., Sebokova J., Ziegelhoffer A. (1997) Mitochondrial contact sites detected by creatine phosphokinase activity in the heart of normal and diabetic rats. Is mitochondrial contact sites formation a calcium-dependent process? Gen. Physiol. Biophys. **16**, 329–338

Final version accepted December 3, 1998