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Identification of creatine kinase isoenzymes in the guinea-pig

Presence of mitochondrial creatine kinase in smooth muscle

Yukisato Ishida[2], Markus Wyss[1], Wolfram Hemmer[1] and Theo Wallimann[1]

[1]*Institute of Cell Biology, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland* and [2]*Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan*

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Isoenzymes of creatine kinase (CK, EC 2.7.3.2) in guinea-pig smooth, cardiac and skeletal muscles as well as in brain were analyzed by cellulose acetate electrophoresis and FPLC gel permeation chromatography. In crude tissue extracts of smooth muscles brain type BB-CK and the hybrid form MB-CK were detected, but in enriched mitochondrial fractions from different guinea-pig smooth muscles, mitochondrial type Mi-CK was unambiguously identified. Smooth muscle Mi-CK displayed the same electrophoretic mobility as Mi-CK from brain, which migrates slower than cardiac Mi-CK. Identical to parallel experiments with Mi-CK from cardiac muscle and brain, smooth muscle Mi-CK could be resolved into dimeric and octameric species, the latter being remarkably stable. In contrast to guinea-pig smooth muscles, Mi-CK was not detected in chicken gizzard tissue extracts nor in enriched mitochondrial fractions thereof. The presence of Mi-CK, predominantly in octameric form, in guinea-pig smooth muscles, but not in chicken gizzard, may represent a clue for the different physiological properties of these muscles and may provide the molecular basis for the dependence of the PCr production on oxidative metabolism observed in the guinea-pig taenia caeci.

Mitochondrial creatine kinase; CK isoenzyme; Smooth muscle of guinea-pig and chicken (aorta, taenia caeci, vas defens); Energetics; Phosphocreatine circuit

1. INTRODUCTION

Smooth muscle, like many other mammalian excitable tissues, contains phosphocreatine (PCr) and ATP, although the PCr content is relatively small compared to that of striated muscles [1]. Already earlier, the corresponding enzyme, creatine kinase (CK, EC 2.7.3.2), was found in smooth muscle of chicken gizzard [2]. After the existence of different CK isoenzymes, i.e. brain-type BB-CK, muscle-type MM-CK and the hybrid form MB-CK had been established [3,4], it was reported that smooth muscle possesses BB-CK and MB-CK [5–10]. Although another isoenzyme, mitochondrial CK (Mi-CK), has been identified in conjunction with cytosolic CK in various mammalian and non-mammalian tissues, e.g. skeletal and cardiac muscle [11], brain [11,12], retina photoreceptor cells [13], spermatozoa [14,15] and *Torpedo* electrocytes [16], little is known about the presence of Mi-CK in smooth muscles except that CK activity was found in the enriched mitochondrial fractions of some smooth muscles [11,17]. On the other hand, recent investigations on PCr and ATP contents of the intestinal smooth muscle showed that the PCr production is highly dependent on the presence of oxygen [17,18], suggesting the presence of Mi-CK in smooth muscles. Therefore, the present study was performed to analyze CK isoenzymes of smooth muscles in comparison to those of cardiac muscle and brain.

Abbreviations: CK, creatine kinase; BB-CK, brain-type CK isoenzyme; MM-CK, muscle-type CK isoenzyme; MB-CK, hybrid form between BB- and MM-CK; Mi-CK, mitochondrial CK isoenzyme; PCr, phosphocreatine; Cr, creatine; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Ap5A, P^1,P^5-di(adenosine-5')pentaphosphate; EDTA, ethylenediamine-tetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid

Correspondence address: Y. Ishida, Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan. Fax: (81) (427) 291252.

2. MATERIALS AND METHODS

2.1. *Extraction of CK isoenzymes from tissues*

Male guinea-pigs (300–500 g) were stunned and exsanguinated. Then, brain (cerebrum), skeletal muscle (psoas), cardiac muscle (ventricle) and smooth muscles (thoracic aorta, vas deferens and taenia caeci) were isolated. Chicken gizzard was obtained promptly after slaughter. CK isoenzymes were extracted by hypo-osmotic swelling of the tissues as described elsewhere [15]. Briefly, tissues were minced, exposed to 1–3 vols. of pure water (Milli-Q, Millipore, Bedford, MA, USA) for 15 min, then incubated with 2–6 vols. of 25 mM NaH_2PO_4 plus 5 mM 2-mercaptoethanol (pH > 8.75) for another 60–90 min, and finally centrifuged at 10000 × g for 10 min. The supernatant served as a tissue extract and was kept at −20°C. The protein concentration was determined by the method of Bio-Rad using bovine serum albumin as a standard and a correction factor of 2.1 for realistic protein content as recommended by the Bio-Rad manual.

2.2. *Mitochondrial fraction*

Mitochondrial fractions of the guinea-pig heart and smooth

muscles were prepared by a modified method of [19]. Isolated tissues were minced in MSH solution of 0.225 M mannitol, 0.075 M sucrose, 0.5 mM EGTA, 2 mM 2-mercaptoethanol and 10 mM Hepes (pH 7.0) and exposed to 100 U collagenase (Type VII, Sigma, St. Louis, MO, USA) for 15 or 60 min to heart or smooth muscles, respectively. Then, the tissues were homogenized by a Polytron (Kinematica, Luzern, Switzerland) or Teflon homogenizer. Homogenates were centrifuged once at 8000 × *g* for 10 min. Pellets were resuspended and centrifuged at 700 × *g* for 10 min. The supernatants were centrifuged twice at 8000 × *g* for 10 min. The resultant pellets were suspended in MSH and served as enriched mitochondrial fractions which were kept at −70°C. For the preparation of guinea-pig brain mitochondrial fraction, the tissue was homogenized without exposure to collagenase, and the homogenate was centrifuged at 700 and 8000 × *g* to get enriched mitochondrial fraction.

2.3. *Extraction of Mi-CK from mitochondria*

Extraction of Mi-CK from mitochondria was performed according to a modified method of [20]. Freshly prepared mitochondria were centrifuged for 15 min at 500 × *g*. The supernatant was centrifuged for 30 min at 27500 × *g*, resuspended and centrifuged again for 40 min at 27500 × *g*. The pellet was swollen by water for 15 min and centrifuged for 40 min at 27500 × *g*. This step was repeated again. The resultant pellet containing mitoplasts was exposed to 25 mM NaH_2PO_4 at pH 9 for 60 min. The phosphate solution was then centrifuged for 80 min at 90000 × *g*. The final supernatant was adjusted to pH 7 and was concentrated with Centricon 10 microconcentrators (Amicon, Danvers, MA, USA). The concentrated sample was subjected to FPLC gel permeation chromatography, measurement of CK activity and cellulose acetate electrophoresis.

2.4. *FPLC gel permeation chromatography*

Gel filtration experiments were performed on an FPLC-SUPEROSE 12 HR 10/30 column (Pharmacia) as described elsewhere [21]. The elution solution was composed of 50 mM NaH_2PO_4, 150 mM NaCl, 2 mM 2-mercaptoethanol, 0.2 mM EDTA and 2 mM NaN_3 (pH 7.2). The column was calibrated with ferritine (440 kDa), catalase (232 kDa), aldolase (158 kDa), chicken BB-CK (80 kDa) and α-chymotrypsinogen (24.5 kDa) as standards. CK activity in each fraction was determined as described below.

2.5. *CK activity measurements*

Using a pH-stat (Radiometer, Copenhagen, Denmark), CK activities in the direction of ATP synthesis were measured by titrating the reaction solution at pH 7 and 25°C, as described previously [22]. One unit of enzymatic activity corresponds to the formation of 1 μmol ATP per min.

2.6. *Cellulose acetate electrophoresis*

Samples prepared from various tissues were applied on cellulose acetate membranes (Cellogel, Chemetron, Milano, Italy) and subjected to electrophoresis for 35–50 min at a constant voltage of 140–150 V in veronal buffer, pH 8.4–8.6, as described elsewhere [20]. Separated isoenzymes were stained by the overlay-gel technique [23] in the presence and absence of 0.01 mM P^1,P^5-di(adenosine-5')-pentaphosphate (Ap5A) which blocks adenylate kinase (AK) activity [24].

3. RESULTS AND DISCUSSION

3.1. *CK activities in total homogenates and mitochondrial preparations of smooth muscle*

CK activities were determined in whole tissue extracts of various organs of the guinea-pig (Table I). On the basis of tissue wet weight, skeletal muscle (psoas) showed the highest specific CK activity tested. Smooth muscles of taenia caeci and vas deferens had specific activities comparable with those of brain (cerebrum) and heart (ventricle), while that of the thoracic aorta was approximately 10 times lower. The low CK activity of aorta may be at least partly due to the larger non-cellular space compared to that of the other tissues. When the activities were expressed in terms of protein content, the above relative proportions of specific CK activities of the various tissues tested were not altered. Table I also shows the CK activity of chicken gizzard smooth muscle which possessed a comparable amount of CK activity as visceral smooth muscles of the guinea-pig.

Table I

Creatine kinase (CK) activities of tissue extracts of the guinea-pig and chicken

Preparations	Activities	
	(U/g wet weight)	(U/mg protein)
Guinea-pig		
Brain	130.1	2.8
Skeletal muscle	2037.8	37.0
Cardiac muscle	251.3	3.9
Smooth muscle		
Taenia caeci	153.3	5.4
Vas deferens	328.7	7.8
Aorta	26.0	0.6
Chicken		
Smooth muscle		
Gizzard	165.1	6.2

The specific CK activities of enriched mitochondrial fractions prepared from heart, brain and smooth muscles were also determined (Table II). Mitochondrial fractions of the guinea-pig heart displayed a specific CK activity of approximately 2 U/mg mitochondrial protein. The cerebral mitochondrial fraction revealed approximately 30% of the cardiac CK activities. In mitochondrial fractions of the taenia caeci and vas deferens, specific CK activities were relatively high, yet slightly lower than that of the cerebral fraction.

Table II

CK activities of enriched mitochondrial fractions prepared from tissues of the guinea-pig and chicken

Preparations	Activities (U/mg mitochondrial protein)
Guinea-pig	
Brain	0.68
Cardiac muscle	2.36
Smooth muscle	
Taenia caeci	0.38
Aorta	0.10
Chicken	
Smooth muscle	
Gizzard	0.10

Mitochondrial fractions of the guinea-pig aorta and chicken gizzard showed only 5% of the activity of the guinea-pig ventricle mitochondria.

3.2. *Identification of CK isoenzymes in smooth muscle*

When smooth muscle tissue extracts were subjected to cellulose acetate electrophoresis and the CK isoenzymes subsequently visualized, 3 bands appeared (Fig. 1). Two bands migrated to the anode and one band migrated slightly to the cathode. The fast moving band towards the anode is BB-CK, since it exactly corresponded to BB-CK from brain (Fig. 1). Such a fast migrating CK band was already reported in smooth muscles [6,7,9]. The second band with slower electrophoretic mobility towards the anode, also reported earlier [5,7,8], is likely to be the hybrid form MB-CK, since it corresponded to the faint band of cardiac muscle tissue extracts [3,24].

The third band of smooth muscle extracts and the faint band at the equivalent place in brain extract seemed to correspond to the intense MM-CK bands of cardiac and skeletal muscle extracts (Fig. 1), but proved to be due to adenylate kinase activity, since it disappeared in the presence of 0.01 mM P^1,P^5-di(adenosine-5')-pentaphosphate (Ap5A), an adenylate kinase inhibitor [24] (Fig. 2). Furthermore, when PCr, a substrate for CK reaction, was omitted from the staining solution in the absence of Ap5A, this very same band appeared at the equivalent place (data not shown), also indicating that it represents adenylate kinase. In contrast, by the same criteria, the cathodic bands in skeletal and cardiac muscle extracts seen in Fig. 1 proved to be largely due to MM-CK and an additional CK isoenzyme, presumably Mi-CK. In brain extracts, two bands could be assigned to Mi-CK. The less cathodic form represents dimeric and the more cathodic form octameric Mi-CK (see below).

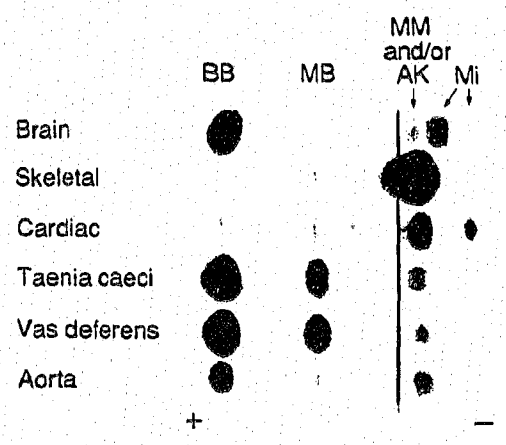

Fig. 1. Cellulose acetate electrophoresis of tissue extracts of guinea-pig brain, skeletal, cardiac and smooth muscles (taenia caeci, vas deferens and aorta). Electrophoresis was performed for 40 min at 145 V. CK isoenzymes were visualized by the overlay gel method [27], in the absence of P^1,P^5-di(adenosine-5')pentaphosphate (Ap5A). BB represents the brain-type BB-CK isoenzyme; MM, the muscle type MM-CK; MB, the hybrid form between MM and BB; and Mi, the mitochondrial CK isoenzyme. AK, adenylate kinase; + and − represent the anodal and cathodal direction, respectively. Sample application was on the vertical line.

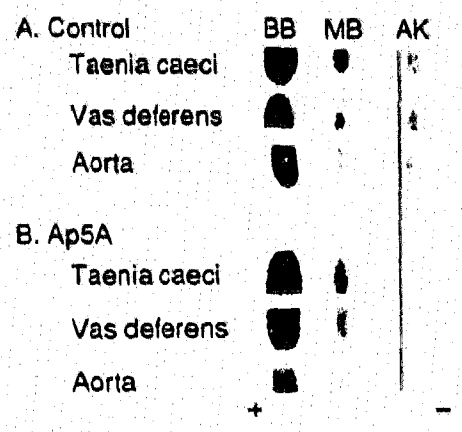

Fig. 2. Effect of Ap5A on the CK zymogram pattern of smooth muscle tissue extracts. A and B, cellulose acetate membrane was stained in the absence and presence of 0.01 mM Ap5A, respectively. Note: in B, bands slightly cathodal near the origin were abolished, indicating the presence of adenylate kinase (AK) at this position. BB and MB represent BB- and MB-CK, respectively. Samples were applied on the vertical line.

In order to obtain confident evidence for the presence of Mi-CK in smooth muscles, enriched mitochondrial fractions of taenia caeci, vas deferens and aorta were subjected to cellulose acetate electrophoresis in comparison to enriched mitochondrial fractions of brain and cardiac muscle (Fig. 3). In these mitochondrial fractions of smooth muscles, some BB-CK and MB-CK were still detected, but clear cathodal bands of Mi-CK appeared in the presence of Ap5A (Fig. 3). The

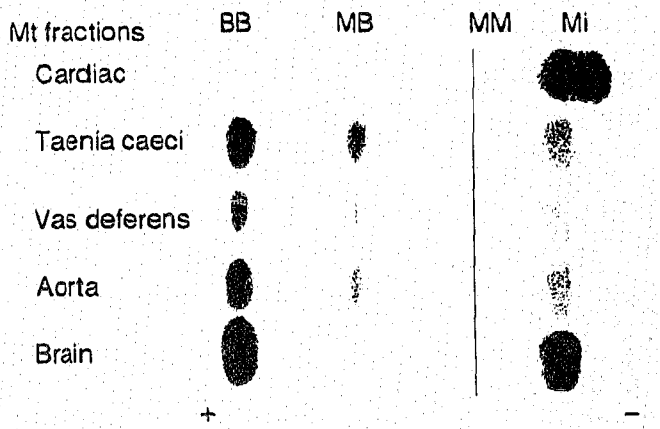

Fig. 3. Mitochondrial CK in smooth muscle. Cellulose-polyacetate electrophoresis of enriched mitochondrial fractions prepared from brain, cardiac muscle, taenia caeci, vas deferens and aorta of the guinea-pig. Membrane was stained in the presence of Ap5A to suppress AK activity. Note: Mi-CK is found in all these smooth muscle preparations.

place of the cathodal band in smooth muscle mitochondria was equivalent to that of the more cathodal band of brain Mi-CK and to that of the less cathodal band of cardiac Mi-CK, but it was distinctly different from that assigned for adenylate kinase (Fig. 3). Omission of PCr from the staining mixture prevented the appearance of all the cathodal bands (data not shown). Therefore, the cathodal band of smooth muscle mitochondrial fractions is indeed due to the activities of Mi-CK.

Chicken gizzard smooth muscle has currently been used for the biochemical and biophysical characterization of its contractile properties. This led us to analyze CK isoenzymes in gizzard as well. Fig. 4 shows the electrophoretic patterns for a whole tissue extract and an enriched mitochondrial fraction of gizzard as well as for a tissue extract of chicken heart. As already known [25], chicken heart showed a band for BB-CK, not MM-CK, and two bands for Mi-CK in the presence of Ap5A. On the other hand, neither tissue extract nor enriched mitochondrial fraction of the gizzard gave any Mi-CK bands, while BB-CK was present in significant amounts. It is thus suggested that, unlike mammalian smooth muscle of the guinea-pig, chicken gizzard smooth muscle does not express Mi-CK.

3.3. *Characterization of smooth muscle MI-CK by FPLC gel permeation chromatography and cellulose acetate electrophoresis*

Mi-CK from different species and tissues is known to exist in dimeric and octameric form [15,20,23,26,27]. To obtain information on the oligomeric state of Mi-CK in the guinea-pig, freshly prepared phosphate extracts of swollen cardiac and cerebral mitoplasts were subjected to gel filtration and the CK activities of the respective fractions were analyzed by the pH-Stat method (Fig. 5). Cardiac phosphate extract revealed

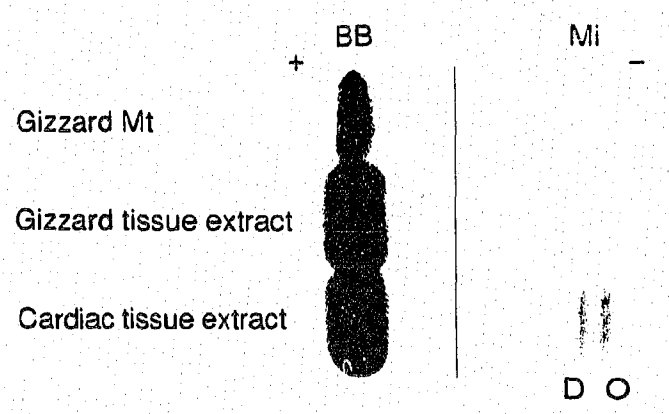

Fig. 4. CK isoenzymes identified in the chicken gizzard smooth muscle. Tissue extract and enriched mitochondrial fraction from gizzard as well as tissue extract of chicken cardiac muscle were subjected to cellulose acetate electrophoresis in the presence of Ap5A to suppress AK activity. Note: complete absence of Mi-CK in chicken gizzard and presence of Mi-CK octamers (O) and dimers (D) in chicken cardiac muscle.

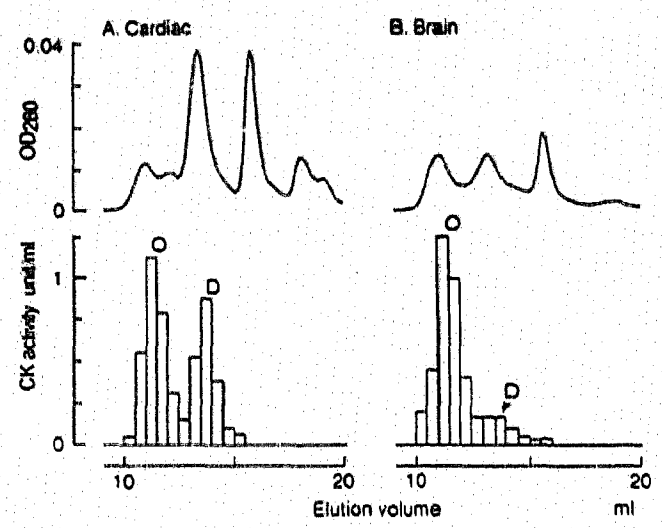

Fig. 5. Gel permeation elution profiles (FPLC Superose 12 chromatography) of phosphate extracts from cardiac muscle (A) and brain (B) mitochondria and CK activity profiles of gel-filtrated fractions. The upper panel shows elution profiles and the lower panel shows the enzymatic activity. O and D refer to the octamer and dimer elution positions of Mi-CK, respectively. Note: the relatively high proportion of Mi-CK dimers in extracts from heart versus brain mitochondria is indicative for a higher stability of brain Mi-CK octamers compared to cardiac Mi-CK octamers.

two peaks of CK activity corresponding to molecular weights of around 85 and 350 kDa, thus being equivalent to those of dimeric and octameric molecules of Mi-CK, respectively [23,28]. On the other hand, in the phosphate extract of brain mitochondria, the 350 kDa peak was predominant and that of 85 kDa was very small. These results suggest that octameric and dimeric Mi-CKs are formed in the guinea-pig cardiac muscle and brain.

Electrophoretic patterns of cardiac and brain mitochondrial phosphate extracts showed two Mi-CK bands in the cathodal area (Fig. 6). Fractions from gel filtration runs containing exclusively dimeric Mi-CK were also included in Fig. 6 for comparison. In two Mi-CK bands of heart and brain, the band for dimeric Mi-CK corresponded to the less cathodic one, the more acidic band therefore representing octameric Mi-CK. The same pattern was observed with the chicken and rat Mi-CK isoenzymes and may be explained by the higher isoelectric point of octameric versus dimeric Mi-CK [20]. As can also be seen in Fig. 6, exclusively octameric Mi-CK was present in brain mitochondria, whereas a mixture of dimeric and octameric Mi-CK was visualized in cardiac mitochondria, eventually signifying that brain Mi-CK octamers are more stable than cardiac Mi-CK octamers.

Furthermore, the brain Mi-CK bands moved slow to the cathodic direction compared to the cardiac Mi-CK bands (Fig. 6). Coincidentally, chicken brain Mi-CK was recently reported to have a slower electrophoretic

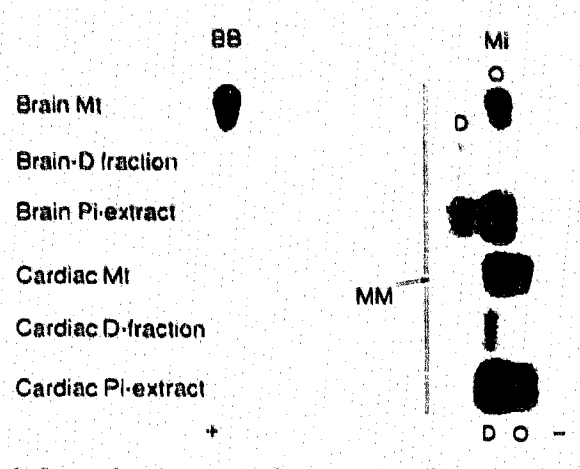

Fig. 6. Comparison between brain-type and cardiac muscle-type Mi-CK isoenzymes of the guinea-pig. Enriched mitochondrial (Mt) fractions, phosphate (Pi) extracts thereof and dimer (D) fractions after gel filtration of the phosphate extracts were subjected to cellulose acetate electrophoresis and bands were visualized in the presence of Ap5A to suppress AK. Note: the different electrophoretic mobility of octamers (O) and dimers (D) of brain Mi-CK versus cardiac Mi-CK. BB, MB, MM and Mi refer to BB-CK, MB-CK, MM-CK and mitochondrial CK.

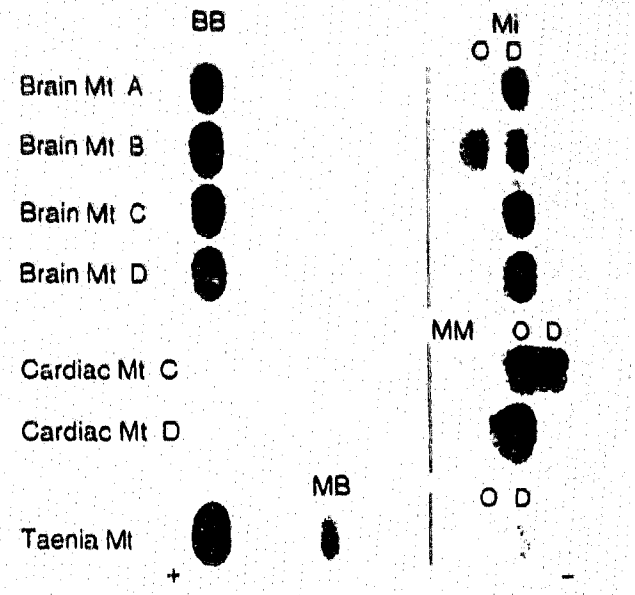

Fig. 7. Effects of dimerizing conditions on electrophoretic patterns of Mi-CK isoenzymes obtained from enriched mitochondrial fractions of brain and cardiac muscle. Dimerizing conditions were made by adding 4 mM ADP, 5 mM $MgCl_2$, 20 mM creatine and 50 mM KNO_3 to a control solution composed of 50 mM NaH_2PO_4, 150 mM NaCl, 0.2 mM EDTA, 2 mM 2-mercaptoethanol and 2 mM NaN_3 (pH 7.2), as reported by [20]. In A, mitochondrial fractions were subjected to freezing-and-thawing 3 times in control solution; B, 3 times freezing-and-thawing in dimerizing conditions; C, mitochondria were exposed for 30 min to control solution at 4°C without repeated freezing-and-thawing; D, exposed for 30 min to dimerizing conditions at 4°C. The pattern of the mitochondrial fraction of taenia caeci after repeated freezing-and-thawing (more than 5 times) in control solution is also shown, indicating that the octameric Mi-CK (O) from smooth muscle, similar to brain Mi-CK octamers, is very stable and is not as easily converted into dimers (D) as cardiac Mi-CK octamers.

mobility than that of the chicken heart on the cellulose acetate membrane [20]. N-terminal sequence analysis revealed that Mi-CKs of chicken brain and heart were two distinct isoenzymes differing in 10 out of 30 N-terminal amino acids [29]. Recently, also in human, two distinct Mi-CK isoenzymes were identified by molecular genetics [30]. These results suggest that mammalians as well as birds possess two distinct isoenzymes of Mi-CK, a brain-type and cardiac muscle-type Mi-CK.

The reversible conversion between Mi-CK dimers and octamers depends on a variety of conditions, such as Mi-CK concentration, pH and the presence of nucleotides and salt [20,31,32]. When mitochondrial fractions of guinea-pig cardiac muscle were exposed for 30 min to dimerizing conditions, the more cathodic band faded in favor of the more anodic band (Fig. 7). On the other hand, the Mi-CK band of the brain mitochondria was apparently not affected by the exposure to dimerizing conditions for 30 min (Fig. 7). However, after repeated freezing and thawing of the brain mitochondria under dimerizing conditions, the original band slightly faded in favor of the more anodic band (Fig. 7). Density ratios of the anodic versus cathodic brain Mi-CK bands were 1.32 after 3 times freezing and thawing under dimerizing conditions and 0.09 after 3 times freezing and thawing under control conditions. These results suggest that in the guinea-pig the octameric form of brain Mi-CK is much more stable than that of the cardiac muscle Mi-CK in the guinea-pig.

Most importantly, smooth muscle Mi-CK was also exposed to dimerizing conditions, but no appreciable change in the density ratio of acidic to basic band was observed on cellulose acetate membranes (data not shown). Instead, several times freezing and thawing during a prolonged storage period of mitochondrial fractions at −70°C gave two bands at comparable positions with those of brain Mi-CK (Fig. 7). Although a direct analysis of the oligomeric state of smooth muscle Mi-CK was not possible, due to the small amounts of Mi-CK and the small amounts of guinea-pig smooth muscle available, the fact that smooth muscle Mi-CK octamers comigrated with brain Mi-CK octamers and, like brain Mi-CK, could partially be converted into Mi-CK dimers proves the fact that smooth muscle Mi-CK can exist as octamers and dimers and that it possesses properties much more similar to brain Mi-CK rather than to cardiac Mi-CK.

The higher stability of brain-type Mi-CK octamers is further suggested by the following facts: among diluted tissue extracts only brain tissue extracts showed an octameric Mi-CK band, although the intensity was relatively weak (Fig. 1). The ratio of Mi-CK octamer to

dimer was much higher in brain compared to cardiac mitochondria (Fig. 3). When phosphate extracts of brain mitochondria were subjected to gel filtration, predominantly the octameric form was detected (Fig. 5). Under physiological conditions the predominant presence of octameric Mi-CK was reported for bovine heart mitochondria, while a predominance of dimeric Mi-CK was found in pigeon breast muscle [32]. In the chicken, cardiac Mi-CK octamers are more stable than brain Mi-CK octamers [20,33]. Thus, species as well as tissue-specific differences in the stability of Mi-CK octamers are obvious.

Table III summarizes the CK isoenzymes detected in tissue extracts and enriched mitochondrial fractions of smooth muscles, cardiac muscle, skeletal muscle and brain of the guinea-pig as well as the chicken gizzard smooth muscle. BB-CK was identified in smooth muscles and tissues other than skeletal muscle. MM-CK was found in skeletal and cardiac muscles of the guinea-pig, but not in other tested tissues including brain. This is in contrast to a recent work in which substantial amounts of MM-CK were found in human brain [34]. This again could point to species differences in the expression of CK isoenzymes.

Table III shows the wide distribution of Mi-CK isoenzymes among tissues except the chicken gizzard smooth muscle. Smooth muscle usually produces long lasting contraction more than several minutes, a so-called tonic contraction. In contrast, it has recently been shown that chicken gizzard responds to the contractile stimuli for a brief period of less than 60 s [35,36]. Presumably, such a difference in the contractile properties of chicken gizzard is at least in part reflected by the lack of Mi-CK in this tissue.

Table III

CK isoenzymes and adenylate kinase (AK) identified in various tissues of the guinea-pig and chicken, using cellulose acetate electrophoresis

Preparations	CK isoenzymes				AK
	BB	MB	MM	Mi	
Tissue extracts					
Guinea-pig					
Brain	+	−	−	+	+
Skeletal muscle	−	+	+	+	+
Cardiac muscle	+	+	+	+	+
Smooth muscle					
Taenia caeci	+	+	−	−	+
Vas deferens	+	+	−	−	+
Aorta	+	+	−	−	+
Chicken gizzard	+	−	−	−	+
Mitochondrial fractions					
Guinea-pig					
Brain	+	−	−	+	nd
Cardiac muscle	−	−	+	+	+
Smooth muscle					
Taenia caeci	+	+	−	+	+
Vas deferens	+	+	−	+	nd
Aorta	+	+	−	+	nd
Chicken gizzard	+	−	−	−	+

BB represents the brain-type BB-CK isoenzyme, MM the muscle-type MM-CK, MB a hybrid form between MM- and BB-CK, Mi mitochondrial CK and AK adenylate kinase. Symbol + means the identification and − the absence of the respective CK isoenzymes and AK; nd, not determined

3.4. *Mitochondrial CK and smooth muscle energetics*

In terms of energy expenditure, both glycolytic and oxidative pathways of metabolism contribute to the function of the guinea-pig taenia caeci. When activated by depolarization, the maximum ATP turnover rate through both pathways of metabolism was calculated to be approximately 5 μmol/min/g wet weight (U/g) at 37°C and approximately 60% of the total turnover rate was due to oxidative phosphorylation [18]. Thus, compared with the total CK activity measured in this muscle (153.3 U/g at 25°C), relatively small amounts of Mi-CK are required to support the PCr production dependent on oxidative phosphorylation. The presence of only small amounts of Mi-CK in smooth muscle may partly explain the failure of detection of Mi-CK in the tissue extracts of guinea-pig smooth muscles. The cytosolic CK activities are suggested to be predominant, while the total CK activities are much higher than the required maximum ATP turnover rate of the taenia caeci. It will be interesting to investigate whether in smooth muscle a certain fraction of 'cytosolic' BB-CK is also compartmentalized and bound to subcellular locations with high energy turnover [37], for CK isoenzymes have been shown to be associated with acetylcholine receptor-rich postsynaptic plasma membranes in *Torpedo* electrocytes [38], to sarcoplasmic reticulum membranes of muscle [39] and to be structurally incorporated into the skeletal and cardiac muscle M-band [40], suggesting a dual structural and enzymatic role.

The presence of Mi-CK in mitochondrial fractions of guinea-pig smooth muscles may provide the molecular basis for the dependence of PCr-production on oxidative metabolism observed in the guinea-pig taenia caeci [17,18]. This is corroborated by the recently reported fact that in the porcine carotid artery, a two site molecular exchange between ATP and PCr due to CK reaction can be assumed from ^{31}P-NMR saturation transfer experiments [41]. Thus, one may postulate that these two corresponding sites in the smooth muscle cell may be cytosolic BB-CK on one hand and mitochondrial Mi-CK on the other hand, forming a so-called PCr-circuit [37] where high-energy phosphate fluxes between mitochondria and cytoplasm are facilitated via Mi-CK and BB-CK. Since the cell volume of smooth muscle is small compared to skeletal muscle [42], diffusion limitation of ATP and ADP may not be as crucial in smooth muscle as in skeletal muscle cells [43] so that the high concentration of BB-CK in these cells, especially in chicken gizzard, may be sufficient for the buf-

fering of ATP/ADP levels attributed to CK [37,44]. However, the presence of appreciable amounts of MI-CK in guinea-pig smooth muscles indicates that under oxidative working conditions the energy transport function of the CK system facilitated via MI-CK [37] becomes physiologically significant in these smooth muscles. Thus, while ATP hydrolysis occurs upon stimulation of smooth muscle contraction, the PCr-circuit system may coordinately work to maintain the cytosolic ADP concentration low, to keep the cytosolic pH constant and on the mitochondrial side to effectively stimulate oxidative phosphorylation in elevating ADP and proton concentrations at the intermembrane space.

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