DEVELOPMENTAL CHANGES IN CREATINE PHOSPHOKINASE ISOENZYMES IN NEONATAL MOUSE HEARTS

by

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SUMMARY

The distribution of creatine phosphokinase isoenzymes differs in extracts of newborn and adult mouse hearts. Electrophoresis on acetate strips reveals the presence of BB, MB, and MM isoenzymes in the 2 day old neonate heart, with relative activities of 4%, 24% and 72% respectively. Beginning at 6 days of age, a fourth isoenzyme, shown to be associated with mitochondria, is seen moving toward the cathode. With age the distribution changes, with BB disappearing by 18 days. By 25 days the relative proportions of MB, MM and mitochondrial CPK have reached 5%, 86% and 9%, respectively, similar to the levels seen in the adult. The late appearance of the mitochondrial isoenzyme may reflect a difference in the requirement of the developing and adult heart for ATP and phosphocreatine.

The existence of multiple molecular forms, isoenzymes, of creatine phosphokinase (CPK) (EC 2.7.3.2) from tissues of higher vertebrates is well documented (1,2,3). The enzyme is a dimer of molecular weight 82,000, and is composed of M or B type subunits. Brain CPK consists of BB isoenzyme. Skeletal muscle CPK consists almost entirely of MM isoenzyme while cardiac tissue contains in addition to MM also some MB hybrid enzyme (4). An additional isoenzyme from mitochondria was reported by Jacobs et al. (5) and the existence of this mitochondrial CPK has since been confirmed by many other investigators. The mitochondrial isoenzyme has been found in the myocardium from rat (5,6), rabbit (7), beef (8), guinea pig and dog (9). The physiological role of these CPK isoenzymes in cellular metabolism is not yet understood. Turner et al have shown that MM CPK is a component of skeletal muscle myofibrils. Fluoroescent antibodies prepared against MM CPK bind to the center of the sarcomere (the M line) (10). Its function in the myofibril is

not known. These authors have also studied the isoenzyme patterns of CPK during development of chick skeletal muscle cells in culture (11). In general, fetal muscle tissue contains the BB isoenzyme which is replaced by MM during development.

In the present study it was found that the mitochondrial isoenzyme is not detected in myocardial tissue of newborn mice or rabbits. It appears about 6 days after birth in the mice. Postnatal changes in the proportions of all of the isoenzymes of the myocardium were followed up to four weeks of age.

MATERIALS AND METHODS

Hearts were removed from animals which had been killed by decapitation or by cervical dislocation. Tissue from human papillary muscles* was kept on ice and extracted within several hours after removal.

Hearts were homogenized in 0.1 M sodium phosphate buffer pH 7.4 containing 2.5 mM β-mercaptoethanol. The homogenate was sonicated for 30 seconds.

Creatine phosphokinase was assayed by the method of Rosalki (12).

Isoenzymes were separated by electrophoresis on Gelman Sephraphore III cellulose acetate strips in tris-barbital buffer, pH 8.8. A volume of homogenate containing 5-10 mIU of CPK was placed on the strip and electrophoresis was carried out at 300 V, 4°C for 2 to 3 hours. The strip was allowed to react with CPK assay reagent (Calbiochem), was dried, and the NADH produced was measured fluorometrically by scanning the strips (13). This method is capable of reproducibly quanitating as little as 0.1 mIU of CPK.

RESULTS AND DISCUSSION

When adult mouse hearts are homogenized in phosphate buffer, sonicated, subjected to electrophoresis, and stained for enzymatic activity, three isoenzymes of CPK are detected (Fig. 1A). The majority of the activity (86%) remains near the origin (MM), about 5% of the total activity moves toward

*Samples of human left ventricular papillary muscles were supplied by Drs. Pat O. Daily and Robert O'Rourke from papillary muscles excised in the course of routine operations for mitral valve replacement.

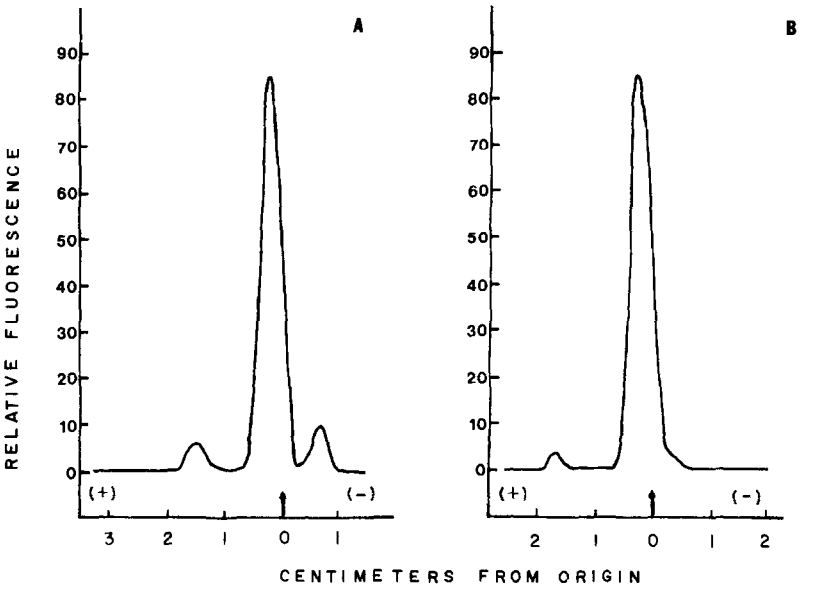

FIGURE 1: Separation of creatine phosphokinase isoenzymes by electrophoresis. Adult mouse heart extracted in (A) 0.1 M sodium phosphate pH 7.5, (B) in 0.25 M sucrose. Electrophoresis and staining as described in methods. Arrows indicate origin.

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the anode (MB), and another minor peak of about 9% moves toward the cathode. This cathodal form has been shown to be mitochondrial in rabbit, rat, and guinea pig, since isolated mitochondrial fractions are enriched with this enzyme. Consistent with this is the absence of the cathodal peak if the hearts are homogenized in 0.25 M sucrose (Fig. 1B).

Analysis of heart extracts from newborn mice or rabbits do not contain any of the mitochondrial isoenzyme. In order to follow the appearance of the mitochondrial enzyme in the mouse, newborn mice were sacrificed at regular intervals after birth, the hearts were extracted in 0.1 M phosphate buffer and the CPK isoenzymes separated and quantitated as described. These results are shown in Fig. 2. Fig. 2A indicates the mitochondrial enzyme is not detectable until about the 6th day after birth and at this time represents only 1% of the total CPK activity. As a percentage of the total it increased steadily

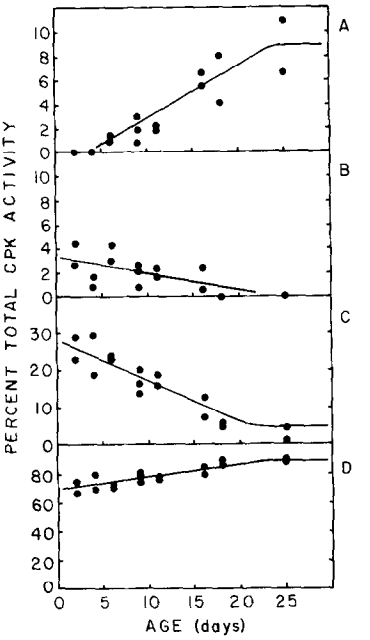

FIGURE 2: Changes in relative proportions of creatine phosphokinase isoenzymes in hearts as a function of time after birth. Each point represents an individual mouse heart. (A) mitochondrial, (B) BB, (C) MB, (D) MM.

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until at 25 days it constituted about 9% of the total activity. The range of mitochondrial CPK found in adult hearts is 7 to 11%, so that at 25 days after birth this isoenzyme has reached adult levels in the mouse.

Changes in the relative level of the other three isoenzymes are also seen during this period. The BB enzyme, which was present at about 3-4% of the total at 2 days after birth, decreased to undetectable levels by 18 days (Fig. 2B). MB was also seen to decrease from 24% to a final value of about 5%, a value similar to that found in adult hearts (Fig. 2C). The MM isoenzyme increased only slightly to about 90% of the total activity (Fig. 2D). By the 25th day following birth the CPK isoenzyme content of the mouse heart is essentially that of the adult.

In an effort to quantitate the amount of mitochondrial CPK in myocardium of other species we assayed tissue from several different animals. These re-

sults are shown in Table 1. With the exception of chicken heart and human papillary muscle, myocardial tissue from all of the species examined contained the cathodal or mitochondrial isoenzyme. It should be noted, however, that Jacobus and Lehninger (14) have reported that CPK activity is associated with mitochondria from human heart. It is possible that the mitochondrial form does exist but is not separated from the MM under these conditions. It seems unlikely that the isoenzyme would be so widely distributed in animals and be absent in man.

Developmental changes in other mitochondrial enzymes have been reported, including prenatal increases in malate dehydrogenase (15) and postnatal increases in cytochrome concentration, the latter probably in response to changes in oxygen availability (16).

Farrell (8) has suggested that the mitochondrial CPK is located on the outside mitochondrial membrane. Jacobus and Lehninger (14) demonstrated that it is outside of the atractyloside sensitive ATP-ADP transport system. However, the physiological function of this isoenzyme still needs to be clarified. It has been suggested by Jacobus and Lehninger (14) and Saks *et al*. (9) that in the presence of creatine, the end product of oxidative phosphorylation is phosphocreatine rather than ATP. One might speculate

TABLE I. QUANTITATION OF ISOENZYMES OF CPK IN MYOCARDIUM

	% TOTAL			
	MM	MB	BB	Mito
Rabbit - 1 day old	85	15	>1%	0
Rabbit - 3 weeks	85	2	0	13
Guinea Pig - Adult	91	3	0	6
Chicken - Adult	0	0	100	0
Human Papillary Muscle	20	80	0	0

that in the fetal and newborn mouse heart, ATP would be needed for synthesis of new cellular constituents and it would not be advantageous to make phosphocreatine at the expense of ATP. As the animal becomes more adult and does not need large amounts of ATP, the mitochondrial CPK is appearing and synthesizing more phosphocreatine relative to ATP. An alternate possibility is that fetal myocardium obtains most of its ATP from glycolysis while the mitochondrial synthesis of ATP becomes important after birth. At this time the mitochondrial CPK has the role of regulating the relative amounts of ATP and phosphocreatine produced.

One other interesting question with regard to the mitochondrial CPK is whether or not it is synthesized in the mitochondria. Experiments are in progress to answer this question.

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