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MITOCHONDRIAL COMPONENT OF THE PHOSPHORYLCREATINE SHUTTLE IS ENHANCED DURING RAT HEART PERINATAL DEVELOPMENT

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Summary. Aerobic metabolism is enhanced during perinatal heart development in parallel with increased cardiac function. The mitochondrial component of the phosphorylcreatine shuttle is important in providing energy for contraction and was examined in weanling and adult rat left ventricle. Creatine kinase activity was enhanced in tissue homogenate and purified cardiac myocytes of adults. Mitochondrial analyses attribute this enhancement to increased creatine kinase activity per milligram mitochondrial protein. Other enzymatic markers of mitochondrial function are not enhanced in activity during perinatal heart growth. The unique response of creatine kinase points to the shuttle mechanism and of mitochondrial creatine kinase, in particular, as a major contributor to heart functional regulation.

Introduction. During perinatal development, rat heart contractile performance is progessively enhanced (1). Because mammalian cardiac function is highly dependent upon aerobic metabolism, it is not surprising that enzymatic estimates of myocardial aerobic metabolism are augmented in a manner paralleling heart function. For example, studies have shown that aerobic metabolic enhancement is confined to cardiac muscle cells with no involvement in nonmuscle cells (2). Additional tissue measurements have documented a marked and progressive increase in specific activities for the mitochondrial marker enzymes citrate synthase and malate dehydrogenase. Comparable marker enzyme activities were unaltered during perinatal heart development when measurements were made in isolated, purified mitochondria (3). Therefore, augmented aerobic metabolism of perinatal heart development is a muscle cell phenomenon which seems to occur via increased numbers of mitochondria per gram tissue without alteration in metabolic activity per mitochondrion.

Abbreviations: ATPase, adenosine triphosphatase (EC 3.6.1.3); CK, creatine kinase (EC 2.7.3.2).

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The above conclusion is based upon commonly employed enzymatic markers; however, recent concepts have emphasized the mitochondrial component of the phosphorylcreatine shuttle in regulating cardiac aerobic energy production. The present study examined the adenosine triphosphatase (ATPase) and creatine kinase (CK) constitutents of the mitochondrial component of the phosphorylcreatine shuttle in the context of the enhanced aerobic metabolism which is known to take place during perinatal heart development.

Methods and Materials. Pregnant, Sprague-Dawley rats gave birth in our animal quarters. At 5 days postbirth, all male rats born on the same day were assigned to dams by random selection in litters of 8 rats/litter. At 21 days of age, rats were either used for experimentation (weanlings) or were weaned, placed in groups of 4 rats/cage and allowed ad libitum food and water until used for experimentation at 9 weeks of age (adults). Weanling and adult rats were killed by a blow to the head. The heart was excised and placed in a beaker of ice-cold saline. For tissue homogenate experiments, atria, great vessels, and connective tissue were dissected from the heart. The right ventricular free wall was removed. Remaining left ventricular tissue (free wall plus interventricular septum) was weighed. A 5% (w/v) homogenate was prepared in 250 mM sucrose buffered with 20 mM Tris, pH 7.6 using a Brinkmann Polytron. Aliquots were removed for subsequent analyses of homogenate protein (biuret) and enzyme activities as outlined below. From the remaining homogenate, mitochondria were isolated by differential centrifugation. Mitochondria were suspended in 100 mM phosphate buffer, pH 7.4, protein measurements (biuret) were made on aliquots of mitochondrial suspensions and, subsequently, enzyme specific activities were measured on additional mitochondrial samples. In separate experiments, retrograde perfusion of the excised heart with a collagenase enzyme solution allowed heart muscle and nonmuscle cells to be separated, with minimal cross contamination, by the methods of Cutilletta et al (4). Homogenous cell fractions were suspended in Tris-buffered sucrose. Aliquots of muscle and nonmuscle cell fractions were used for DNA determination (diphenylamine) and for enzyme analyses as outlined below.

Tissue homogenate, muscle and nonmuscle cell fractions and isolated mitochondria were analyzed for CK activity using the procedure of Rosalki (5). Mitochondrial ATPase activity was measured in a reaction mixture containing 1mM $MgSO_4$, 20mM Tris, pH 7.4, 1mM Na_2ATP and approximately 1.5 mg mitochondrial protein. Other mitochondrial aliquots were analyzed for malate dehydrogenase, citrate synthase and 3-hydroxyacyl-CoA-dehydrogenase enzyme activities (2).

Results. Enzyme results related to homogenate and muscle and nonmuscle cells are given in Figure 1. CK activity per milligram protein (left panel) indicates a generalized, growth-related increase in enzyme activity within left ventricular tissue. Enhanced CK activity is limited to cardiac muscle cells in view of the substantial increase per milligram DNA within this cell population (right panel). It is also interesting to note the much lower CK activity in nonmuscle cells of weanling left ventricle. As perinatal heart growth transpires, muscle cell CK activity is considerably enhanced while nonmuscle cell activity is reduced.

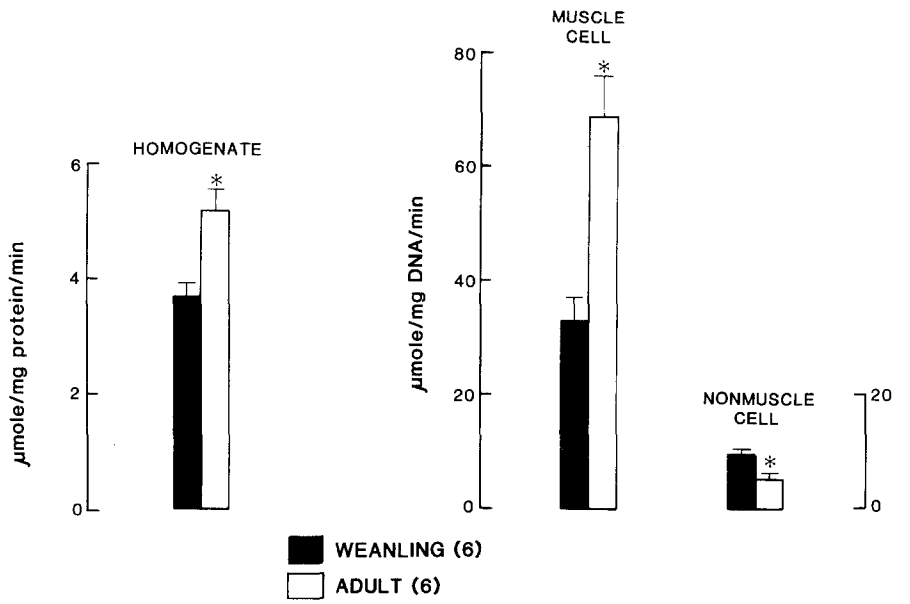

Figure 1. CK enzyme activities in weanling and adult left ventricular homogenate and in cardiac muscle and nonmuscle cells. Number of preparations is given in parentheses. *P<0.05 versus weanling.

The mitochondrial enzymes, ATPase and CK, form two integral parts of the enrgy-producing segment of the phosphorylcreatine shuttle. Therefore, it was of interest to examine ATPase and CK enzymes in identical isolated mitochondria (Figure 2). Mitochondrial ATPase activity is not significantly augmented during

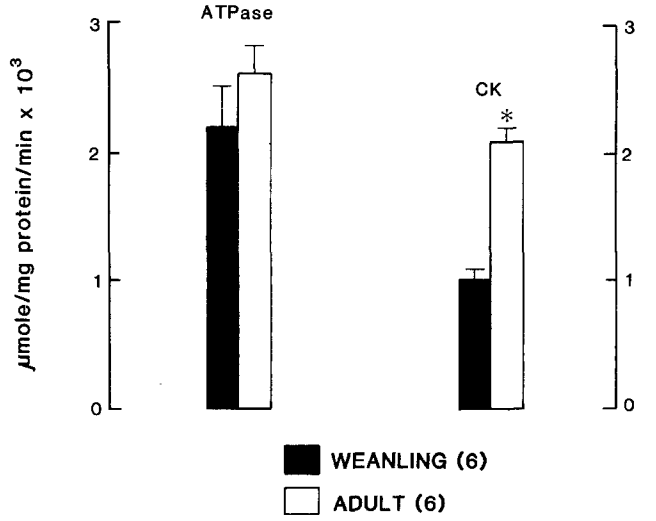

Figure 2. ATPase and CK enzyme activities in isolated mitochondria from weanling and adult left ventricle. Number of preparations is given in parentheses. *P<0.05 versus weanling.

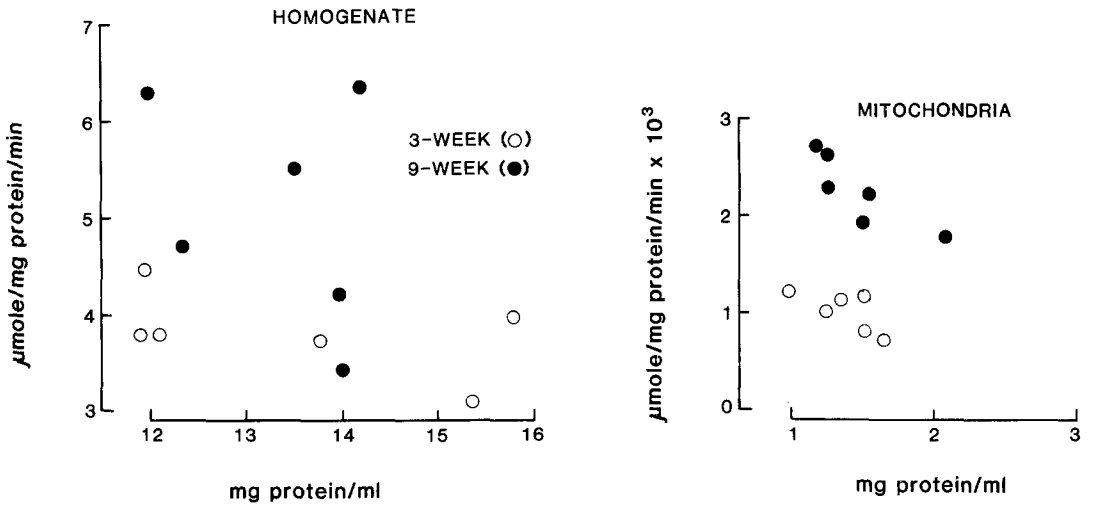

Figure 3. CK enzyme activity in homogenate and mitochondria from weanling and adult left ventricle as a function of protein concentration in assay.

normal, perinatal left ventricular development. In contrast, specific activity of mitochondrial CK increases nearly two-fold during the weanling-adult transition. Because CK activity measurements are known to be markedly influenced by enzyme dilution, both homogenate and mitochondrial enzyme activities are given as functions of enzyme assay protein concentration in Figure 3. Homogenate CK activity exhibited a strong negative correlation with protein concentration (left panel); however, enhanced CK activity is evident when adult values are compared with weanling values. On average, protein concentrations were not significantly different between adult (13.65±.56mg protein/ml; mean ± SE) and weanling (13.20±.60) homogenate samples.

Mitochondrial CK activity also exhibited a negative correlation with protein concentration (right panel), but enhanced mitochondrial specific activity is clearly shown with adult versus weanling heart mitochondria. Mitochondrial protein concentrations were not significantly different between adult (1.49±.13 mg protein/ml; mean ± SE) and weanling (1.40±.09) mitochondrial samples.

Isolated mitochondria utilized for ATPase and CK measurements (Fig. 2) were concomitantly assayed for additional metabolic marker enzymes (Fig. 4). No significant alteration in enzyme marker activity (malate dehydrogenase, citrate

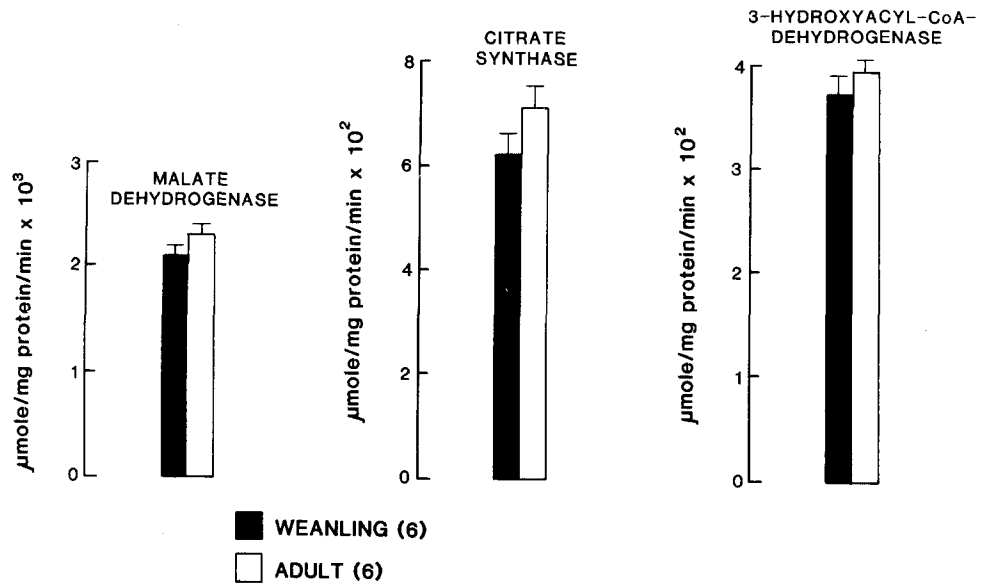

Figure 4. Mitochondrial marker enzyme activities in isolated mitochondria from weanling and adult left ventricle. Number of preparations is given in parentheses and are identical preparations assayed for results given in Figure 2.

synthase and 3-hydroxylacyl-CoA-dehydrogenase) was noted between heart mitochondria isolated from weanling and adult left ventricular tissue.

Discussion. Previous experiments have established enhanced aerobic metabolism as a corresponding perinatal heart characteristic accompanying augmented cardiac function. Increased aerobic metabolism is confined to cardiac muscle cells and enhanced aerobic metabolism occurs via disproportionately increasing numbers of mitochondria with respect to expanding tissue mass. On the basis of enzymatic markers studied previously, the metabolic capacity of a given mitochondrion does not change during the transition from weanling to adult.

The mitochondrial component of the phosphorylcreatine shuttle is considered an important aerobic mechanism supplying metabolic support for cardiac muscle contraction (6). Mitochondrial ATPase is responsible for rephosphorylating ADP to the high energy compound ATP. In turn, ATP is then utilized as a high energy phosphate donor by mitochondrial CK to regenerate creatine phosphate from creatine. Based on whole homogenate results, the process of heart transition from weanling to adult is accompanied by enhancement of overall CK activity. Furthermore, analyses of muscle and nonmuscle cell fractions clearly show CK

enzymatic enhancement is occurring exclusively within cardiac muscle cells. In these respects, the observed CK results are consistent with those observed for other components of aerobic metabolism (2,3) From the homogenate and muscle cell results, no distinction can be made regarding the potential component(s) or compartment(s) contributing to the increased CK enzymatic activity. For this reason, additional measurements were performed on isolated mitochondria. Results show a different response pattern for two key enzymes of the mitochondrial component of the phosphorylcreatine shuttle. Mitochondrial ATPase specific activity is unchanged during perinatal heart development while, in contrast, mitochondrial CK specific activity is dramatically increased. Interestingly, other marker enzymes measured in identical mitochondrial preparations show no alterations in specific activity.

Functional differences in the above mitochondrial enzyme activity responses must be considered. Malate dehydrogenase and citrate synthase are enzymes of the tricarboxylic acid cycle. Hydroxyacyl-CoA-dehydrogenase is associated with fatty acid metabolism, while ATPase activity reflects oxidative phosphorylation in addition to its association with mitochondrial CK as a shuttle component. Location of the enzymes within mitochondrial compartments should also be mentioned. Citrate synthase, malate dehydrogenase, and hydroxyacyl-CoA-dehydrogenase are all matrix enzymes while mitochondrial ATPase is located within the inner mitochondrial membrane. In contrast, mitochondrial CK is positioned on the outer surface of the inner mitochondrial membrane and, thus, occupies a distinctive microanatomical location with respect to the other enzymes assayed.

Although metabolic functions and microanatomical locations differ for the mitochondrial marker enzymes under investigation, their mode of cellular synthesis is identical. The mitochondrial enzymes studied are encoded on the nuclear genome, synthesized within the cell via cytoplasmic ribosomes, and then transported and incorporated into the appropriate mitochondrial location (7). With the exception of CK, all enzymes studied require transport across both boundary membranes and incorporation within the mitochondrion. However, CK, by virtue of its distinctive location, may be transported and/or processed

differently, thus, contributing to enhanced specific activity within the mitochondrion.

The operational mechanisms leading specifically to enhanced mitochondrial CK activity during perinatal heart developemnt are unknown. Nevertheless, the two-fold enhancement of creatine kinase specific activity is unique and provides the potential for assuring improved aerobic production of high energy compounds required for heart contractile performance. The specific and unusual response of CK as a mitochondrial component of the phosphorylcreatine shuttle during perinatal heart development points to the consideration of the shuttle mechanism and of mitochondrial CK, in particular, as a major contributor to heart functional regulation.

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