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ACTIVITY OF PHOSPHORYLCREATINE SHUTTLE ENZYMES IN RAT CARDIAC, FAST-, AND SLOW-TWITCH SKELETAL MUSCLES

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Summary. Energy producing and energy utilizing reactions in muscle are coherently linked by a phosphorylcreatine shuttle mechanism. Operational components of the shuttle were evaluated in muscles encompassing a wide metabolic and contractile spectrum. Consistent CPK/ATPase ratios suggest homogeneous function of the myofibrillar component of the shuttle. Mitochondrial CPK/ATPase ratios vary due to 4-fold differences in CPK activity with respect to oxidative phosphorylation. The phosphorylcreatine shuttle mechanism, and the mitochondrial component in particular, seems a relevant, physiological regulator of skeletal and cardiac muscle function.

Introduction. The classic work of Barany (1) established the direct relationship between skeletal muscle shortening velocity and contractile protein adenosine triphosphatase (ATPase) enzyme activity. Cardiac muscle contractile protein ATPase activity and heart contractile function are also intimately related (2). Generally applicable, function-biochemical correlations markedly advanced the understanding of muscle function; however, current concepts (3,4) show that more complex biochemical reactions must now be incorporated into the muscle function scheme.

According to the phosphorylcreatine (PC) shuttle mechanism proposed by Bessman et al. (3), transfer of energy from mitochondria to cytosol is mediated via creatine phosphate. At the mitochondrial level, oxidative phosphorylation yields the high-energy phosphate substance, ATP, which is then available for the rephosphorylation of creatine via mitochondrial creatine kinase (CPK). Creatine phosphate then diffuses into the cytosol as an "energy source" for muscular contraction. At the contractile protein level, enzymatic reactions similar to those in mitochondria comprise the major energy utilizing component of muscle. CPK bound to contractile protein transfers the high-energy phosphate moiety from creatine phosphate to ADP, thus regenerating ATP. Contractile protein ATPase then hydrolyzes

Abbreviations: ATPase, adenosine triphosphatase (EC 3.6.1.3); CPK, creatine phosphokinase (EC 2.7.3.2); PC, phosphorylcreatine.

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its substrate (ATP), by mechanisms understood in extensive detail, to initiate contractile processes.

Mechanisms responsible for muscle function involve energy producing (mitochondrial) and/or energy utilizing (contractile protein) reactions. Moreover, interrelationships within and between these systems seem likely to exert additional controls upon muscle function. Because the PC shuttle mechanism possesses control features, the potential physiological contribution of the shuttle mechanism was examined.

Methods and Materials. Adult, male Sprague-Dawley rats were used. Animals were weighed, anesthetized with pentobarbital, placed on positive-pressure ventilation with room air, and a mid-sternal thoracotomy was performed. After 5-10 minutes of ventilation, the heart was excised and placed in a beaker in ice. Tibialis anterior and soleus muscles were excised from both hindlimbs and placed in a beaker in ice. Atria, great vessels, and connective tissues were dissected from the heart. The right ventricular free wall was removed. Remaining left ventricular tissue (free wall plus interventricular septum) was weighed. Skeletal muscle samples were weighed after connective tissue and tendon had been removed. Tissue homogenates (5%, w/v) were prepared in 250 mM sucrose buffered with 10 mM Tris, pH 7.4. Aliquots were removed for subsequent analyses of homogenate protein (biuret) and CPK enzyme activity. After centrifuging the remaining homogenate at 600 x g for 10 minutes, the supernatant was decanted and saved. Precipitate from the initial centrifugation was washed once with buffered sucrose. Supernatant from the wash was combined with the original supernatant. Myofibrils were isolated from the washed precipitate (5) and suspended in 50 mM Tris, pH 7.4 which contained 150 mM KCL. Protein concentration was measured (biuret) and, subsequently, adjusted to 6 mg/ml. Myofibrillar protein was stored overnight at 2-5°C until assayed for ATPase and CPK enzyme activities. Mitochondria were isolated from the combined supernatant of the initial myofibril purification steps by differential centrifugation procedures (6). Mitochondria were suspended in buffered sucrose. Aliquots were used to measure mitochondrial protein content (7), ATPase, and CPK enzyme activities.

Magnesium-stimulated, myofibrillar ATPase was measured using the procedures previously described (5). Mitochondrial ATPase was measured in a reaction mixture containing 2 mM $MgSO_4$, 20 mM Tris, pH 7.4, 2mM Na_2ATP, and 250 ug of mitochondrial protein in a final volume of 4.0 ml. The reaction was initiated by substrate (ATP) addition. After incubation for 2 minutes at 30°C, the reaction was stopped with 1 ml of 10% trichloroacetic acid. Precipitated protein was removed by centrifugation and the supernatant fluid was assayed for inorganic phosphate (8). All CPK determinations were by the method of Rosalki (9). Tissue homogenates and mitochondrial samples were treated with Triton X-100 to fully activate CPK prior to assay at 30°C.

Results. Protein and CPK enzyme results for tissue homogenates are shown in Figure 1. Protein concentrations were similar in the three tissues; therefore, homogenate enzyme activities are comparable when expressed either per gram tissue or per milligram protein. Total CPK activity was significantly higher in both soleus and tibialis anterior skeletal muscle with respect to left ventricular tissue. Tibialis anterior muscle, in turn, exhibited substantially higher CPK activity than soleus muscle.

Mitochondrial ATPase and CPK specific activities are given in Figure 2. ATPase fluctuations are apparent among the tissues; however, none of the observed differences were statistically significant. In contrast to ATPase,

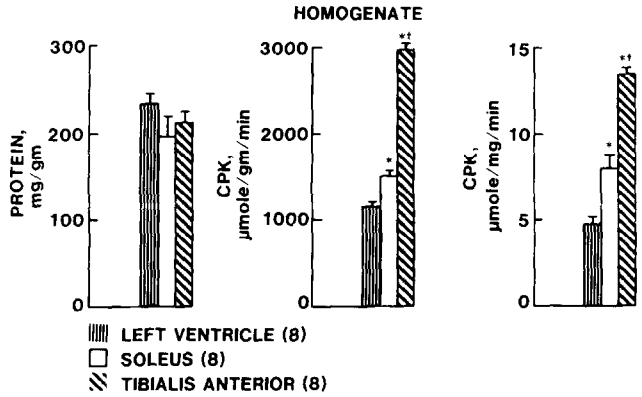

Figure 1. Protein concentration and CPK enzyme activities in muscle tissue homogenates. Values shown are mean ± SE. Number of tissue samples is given in parentheses. *P<0.05 versus left ventricle. †0.05 versus soleus.

CPK activity was 3-4-fold greater in skeletal muscles than in cardiac muscle. Mitochondria from soleus muscle exhibited higher CPK activity than mitochondria from tibialis anterior muscle. Mitochondrial CPK/ATPase activity ratios (Fig. 2) qualitatively reflect the CPK activity results for the tissues.

Myofibrillar enzyme results are shown in Figure 3. In keeping with the known contractile properties of the muscles studied, a 5-fold range of myofibrillar ATPase was observed. Slow-twitch skeletal muscle (soleus) possessed the lowest ATPase activity while fast-twitch skeletal muscle (tibialis anterior) exhibited the highest ATPase values. Ventricular cardiac muscle had intermediate ATPase values. Myofibrillar CPK activities were qualitatively comparable to the ATPase results. Myofibrillar CPK/ATPase values ranged from 2.2

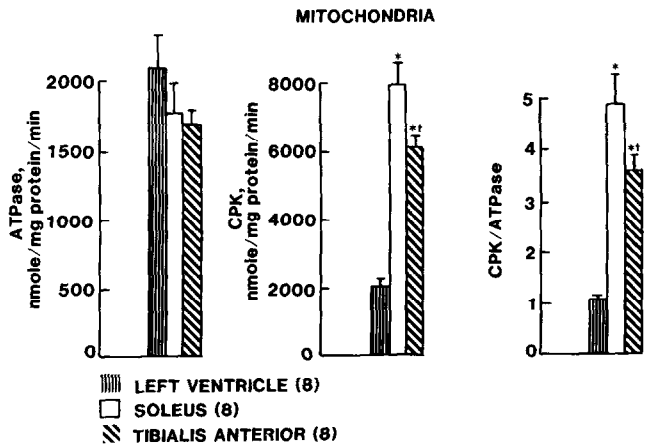

Figure 2. ATPase, CPK, and CPK/ATPase enzyme activity values in isolated mitochondria. Values shown are mean ± SE. Number of tissue samples is given in parentheses. *P<0.05 versus left ventricle. †P<0.05 versus soleus.

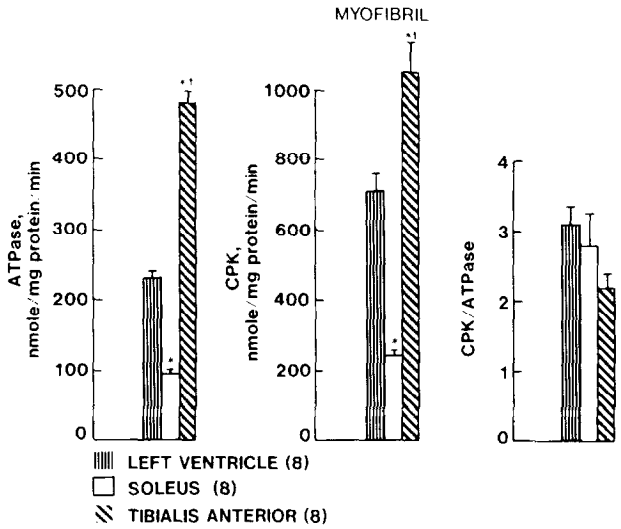

Figure 3. ATPase, CPK, and CPK/ATPase enzyme activity values in purified myofibrils. Values shown are mean ± SE. Number of tissue samples is given in parentheses. *P<0.05 versus left ventricle. †P<0.05 versus soleus.

(tibialis anterior), to 2.8 (soleus), to 3.1 (left ventricle), but differences among the muscles were not statistically significant.

Mitochondria/myofibril comparisons for ATPase and CPK activities are shown in Figure 4. Mitochondrial activities of ATPase and CPK are considerably higher than the respective enzyme activities measured in myofibrils regardless of the type muscle examined. Mitochondria/myofibril values for ATPase and CPK are significantly higher in soleus muscle than in left ventricular cardiac muscle. With respect to soleus muscle, these values are significantly lower in tibialis anterior muscle. Divergent tibialis anterior characteristics were apparent versus left ventricular values. Significantly lower or higher levels were observed for mitochondria/myofibril ATPase and CPK activities, respectively, when compared with cardiac muscle.

Discussion. Cellular mechanisms responsible for muscle contractile function involve energy producing (mitochondrial) and/or energy utilizing (contractile protein) reactions. Moreover, interrelationships within and between these systems seem likely to influence muscle function. Recently, a PC shuttle mechanism was proposed to link energy producing with energy utilizing reactions. Aspects of the shuttle mechanism have profound implications. Mitochondria synthesize creatine phosphate more effectively from ATP generated by oxidative phosphorylation than from cytosolic ATP (10). Therefore, coordinated activity of mitochondrial ATPase and mitochondrial CPK is essential to channel energy, in the form of creatine phosphate, from mitochondria to myofibrils. ATP produced by oxidative phosphorylation is not directly used as

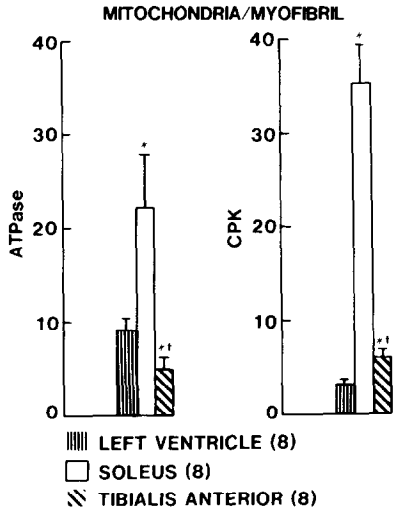

Figure 4. Relative enzymatic activities of ATPase and CPK in mitochondria/myofibril. Values shown are mean ± SE. Number of tissue samples is given in parentheses. *P<0.05 versus left ventricle. †P<0.05 versus soleus.

substrate in contractile protein ATPase reactions because myofibrils preferentially hydrolyze ATP generated, by myofibrillar CPK, from cytosolic ADP and creatine phosphate (10). Contractile protein ATPase and myofibrillar CPK are arranged so that regenerated ATP is compartmentalized to gain rapid and selective access to the active site of ATPase in a cyclic manner.

With the above considerations in mind, tissue homogenate, purified myofibrils, and isolated mitochondria were analyzed in rat muscle tissues selected to encompass a wide spectrum of metabolic and contractile properties. Properties varied from low aerobic metabolism-fast contractile speed (tibialis anterior) to high aerobic metabolism-slow contractile speed (soleus). Enzyme activities are, in general, consistent with the known metabolic (homogenate CPK and mitochondrial CPK) and contractile (myofibrillar ATPase) properties of the several tissues. Large variations exist for the interrelationships within and between the PC shuttle components. Despite a 5-fold difference in myofibrillar CPK specific activity between slow- and fast-twitch skeletal muscles, rather consistent CPK/ATPase ratios reflect a homogeneous operational level of the myofibrillar component of the PC shuttle mechanism. An enzymatic index of oxidative phosphorylation (mitochondrial ATPase) is not significantly different in the various muscle tissues, but a 4-fold difference in mitochondrial CPK activity suggests substantial variation in functional level of the mitochondrial component of the shuttle mechanism. Varying mitochondrial CPK/ATPase ratios in different muscle tissues illustrate this operational principle. The mitochondria/myofibril ATPase ratio reflects the relationship between energy production and

energy utilization. The mitochondria/myofibril CPK ratio represents the relative operational level of the two components specifically involved in the PC shuttle. Mitochondria/myofibril values encompass a range from 3.0 to 32.5. Disproportionately high levels of energy producing reactions versus energy utilizing reactions occur in slow-twitch skeletal muscle. In contrast, muscles having faster speed of contraction are supported by more "efficient" energy producing reactions. The operational level of mitochondrial CPK with respect to oxidative phosphorylation seems to exert a particularly strong influence on muscle functional characteristics. Thus, the PC shuttle mechanism requires consideration as a relevant, physiological regulator of normal skeletal muscle and normal cardiac muscle function.

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