ROLE OF CREATINE IN THE REGULATION
OF CARDIAC PROTEIN SYNTHESIS

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The observation that increased muscular activity leads to muscle hypertrophy is well known, but identification of the biochemical and physiological mechanisms by which this occurs remains an important problem. Experiments have been described (5, 6) which suggest that creatine, an end

product of contraction, is involved in the control of contractile protein synthesis in differentiating skeletal muscle cells and may be the chemical signal coupling increased muscular activity and increased muscular mass. During contraction, the creatine concentration in muscle transiently increases as creatine phosphate is hydrolyzed to regenerate ATP. In isometric contraction in skeletal muscle for example, Edwards and colleagues (3) have found that nearly all of the creatine phosphate is hydrolyzed. In this case, the creatine concentration is increased about twofold, and it is this transient change in creatine concentration which is postulated to lead to increased contractile protein synthesis. If creatine is found in several intracellular compartments, as suggested by Lee and Visscher (7), local changes in concentration may be even greater then twofold. A specific effect on contractile protein synthesis seems reasonable in light of the work of Rabinowitz (13) and of Page et al. (11), among others, showing disproportionate accumulation of myofibrillar and mitochondrial proteins in response to work-induced hypertrophy and thyroxin-stimulated growth.

Previous experiments (5, 6) have shown that skeletal muscle cells which have differentiated in vitro or in vivo synthesize myosin heavy-chain and actin, the major myofibrillar polypeptides, faster when supplied creatine in vitro. The stimulation is specific for contractile protein synthesis since neither the rate of myosin turnover nor the rates of synthesis of noncontractile protein and DNA are affected by creatine. The experiments reported in this communication were undertaken to test whether creatine selectively stimulates contractile protein synthesis in heart as it does in skeletal muscle.

MATERIALS AND METHODS

Fetal Mouse Heart Organ Culture

Isolated hearts from 17- to 21-day fetal mice maintained in organ culture were chosen as the experimental model for this study. Details of the preparation have been described elsewhere (16). Briefly, intact beating hearts from fetal mice were maintained on stainless steel grids at a gas (95% O_2 + 5% CO_2)-medium interface in shallow culture chambers at 37°C. Hearts were cultured for 2-4 days in medium 199 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with insulin (50 μg/ml, Sigma Chemical Co., St. Louis, Mo.) with or without creatine (5 mM, Calbiochem Co., La Jolla, Calif.)

Rates of Protein Synthesis

Rates of synthesis of total protein and myosin heavy-chain were measured as previously described (5, 6). Hearts were incubated for 4 h with L-[4,5-³H]leucine, 50 Ci/mmol (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.), in leucine-free medium 199 and then incubated for 1 h with nonradioactive leucine-containing medium 199. The incorporation of leucine into total protein was determined by measuring the amount of label (counts per minute per milligram wet weight) in aliquots of the total cell homogenate, or into trichloroacetic acid-insoluble material in such aliquots.

The remaining cell homogenate was used to measure the rate of myosin heavy-chain synthesis, employing the method of Paterson and Strohman (12) for in vitro muscle preparations. This method uses disc gel electrophoresis as a micropreparative tool to isolate myosin heavy-chain from tissue homogenates. The myosin heavy-chain band was identified by comparison with reference gels of purified rabbit and chick skeletal muscle myosins. As shown in Fig. 1, a single band migrating ca. 10 mm from the top of the 4% polyacrylamide gel can be visualized upon staining with Coomassie blue even from as little as 2-3 mg wet weight of fetal mouse heart tissue. The upper 15 mm of the gel was sliced into 1-mm thick disks and the counts per minute associated with disks containing the myosin heavy-chain band was determined by counting in Protosol (Amersham/Searle Corp., Arlington Heights, Ill.)-toluene/2,5-diphenyloxazole/1,4-bis[2-(5-phenyloxazole)]benzene (POP/POPOP) in a scintillation counter. The amount of labeled precursor in the region of the gel containing myosin heavy-chain (counts per minute per myosin heavy-chain band/milligram wet weight of muscle) is proportional to the number of myosin molecules synthesized during the pulse, i.e. it is a measure of myosin heavy-chain synthesis (10, 12).

Other Analyses

In other experiments, hearts cultured in the presence or absence of creatine were homogenized and assayed for protein content (8) and for the following enzyme activities: creatine phosphokinase (14), lactic dehydrogenase (2), acid phosphatase (1, 17), and cathepsin D (1, 17).

In all experiments hearts supplied with creatine were compared to hearts of matched littermates cultured under identical conditions in the absence of creatine. The average litter contains 8-12 fetuses. Data obtained from two to six hearts cultured in the presence of creatine (randomly selected at the time of explantation) were compared to data from two to six littermate hearts cultured in the absence of creatine for each experiment reported (n = 7-32 as shown in Table I). Data are expressed as the mean ± 1 standard error, and statistical analyses were made using Student's t-test for paired data.
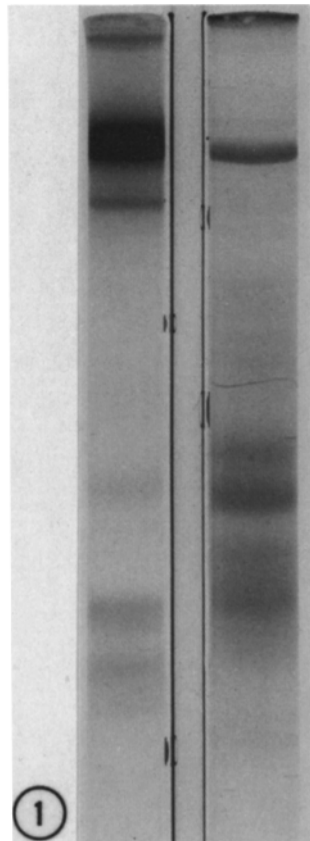
FIGURE 1 Gel electrophoresis patterns of rabbit skeletal muscle myosin (left) and fetal mouse heart myosin (right). 4% SDS-urea polyacrylamide gels were used to isolate myosin heavy-chain from homogenates of fetal mouse heart (in this case from 3 mg wet weight). Myosin heavy-chain migrates ca.10 mm from the top of the gel. The region of the gel containing myosin heavy-chain was sliced into 1-mm thick slices and counts per minute associated with those disks containing the protein band were determined by counting in Protosol-toluene/PPO/POPOP (see Materials and Methods).

RESULTS

The effect of creatine on the rates of synthesis of total protein and of myosin heavy-chain is summarized in Table I. The incorporation of leucine into myosin heavy-chain, the major myofibrillar protein, isolated from hearts supplied with creatine for 2 days was significantly greater (21%) than in control hearts. On the other hand, incorporation of leucine into the cardiac cell homogenate (i.e.,

contractile plus noncontractile protein) was only 6% greater in hearts supplied with creatine.

The activities of several enzymes were also compared in hearts supplied with and without creatine. In hearts supplied with creatine, the activity of creatine phosphokinase, a muscle-specific enzyme localized in part in the myofibril (15), was stimulated 14%, while the activities of several nonmyofibrillar enzymes, viz. glycolytic and lysosomal enzymes, were unaffected (Table I). Cardiac protein content in hearts supplied with and without creatine is also given in Table I; hearts supplied with creatine show a small but significant increase in protein content.

DISCUSSION

These results suggest that myofibrillar protein synthesis in cardiac muscle is selectively enhanced by creatine. The relatively small but significant increases in the rate of total protein synthesis and in total protein content compared to the larger increases in the rate of myosin heavy-chain synthesis and in creatine phosphokinase activity reflect an increase in one subgroup of cellular proteins, viz. the muscle-specific proteins, with little or no change in the nonspecific proteins. Contractile or muscle-specific proteins constitute only a small fraction of total cardiac protein in immature fetal hearts; this is also the case in chick embryo skeletal muscle differentiating in vitro (5) and in ovo (4).

Increased incorporation of labeled precursor into protein could, of course, result not only from increased synthetic rates but also from an expansion of amino acid pool sizes and/or alterations in the rates of protein turnover. Even if these were to occur, however, neither the preferential stimulation of the rate of myosin synthesis nor the selective increase in creatine phosphokinase activity could be explained. Rather, some other mechanism must be functioning in the regulation of muscle-specific protein synthesis by creatine. Creatine could function as a transcriptional or translational factor, or may act to alter levels of charged tRNA's or amino aicd pools which may be specific for myofibrillar protein synthesis, or to alter assembly or degradation of myofibrils. Whatever the mechanism, the results presented in this report suggest that creatine specifically alters the synthesis and accumulation of muscle-specific proteins in heart, as in skeletal muscle. The preferential increase in muscle-specific proteins is consist-

TABLE I

The Effects of Creatine on the Incorporation of Leucine into Cardiac Protein and on Cardiac Enzyme Activities

	Control cultures	Cultures supplied with creatine	Difference	Percent of control
1. Incorporation of radioactive leucine into total cardiac protein (cpm/mg wet weight) (n = 22)	25,600 ± 1,260	27,200 ± 1,580	+1,600 ± 670*	106
2. Incorporation of radioactive leucine into myosin heavy-chain (cpm/mg wet weight) (n = 22)	730 ± 109	880 ± 117	150 ± 43‡	121
3. Total cardiac protein content (mg/heart) (n = 32)	0.260 ± 0.012	0.273 ± 0.013	+0.013 ± 0.004‡	105
4. Creatine phosphokinase activity (μmol ATP/ min/mg protein) (n = 32)	0.856 ± 0.030	0.972 ± 0.029	+0.116 ± 0.022‡	114
5. Lactic dehydrogenase activity (μmol NADH/ min/mg wet weight) (n = 21)	0.130 ± 0.009	0.137 ± 0.009	+0.007 ± 0.004	105
6. Acid phosphatase activity (nmol nitro-phenol/h/mg protein) (n = 8)	307 ± 23	309 ± 26	+2 ± 7	101
7. Cathepsin D activity (μg tyrosine/h/mg protein) (n = 7)	69.6 ± 6.8	67.4 ± 7.0	−2.2 ± 2.1	97

Hearts from matched littermates were cultured for 2–4 days in the presence or absence of 5 mM creatine and assayed as described in the text. The number of experiments performed (n) is given after each entry; each experiment compared two to six hearts. Values are expressed as the mean ± 1 standard error. Values given for the difference were calculated using Student's t-test for paired data.
* $P < 0.05$.
‡ $P < 0.01$.

ent with the observations of Morkin et al. (9) studying work-induced cardiac hypertrophy in vivo. These workers observed greater than 100% stimulation in myosin synthesis while total protein synthesis was increased only about 50%. However, extrapolation of our results obtained with fetal tissue to the adult situation can be made only with great caution; an experimental test of the effect of creatine on muscle-specific protein synthesis in mature muscle remains to be done.

SUMMARY

These experiments indicate that creatine, an end product of contraction unique to muscle, stimulates muscle-specific protein synthesis in the intact beating fetal mouse heart in organ culture. In hearts supplied with creatine (5 mM), the incorporation of labeled precursor into myosin heavy-chain, the major contractile protein, was stimulated 21% and the activity of creatine phosphokinase, a muscle-specific enzyme, was stimulated 14%. In contrast, the incorporation of labeled precursor into total cardiac protein was stimulated only 6% and the activities of several nonmyofibrillar enzymes (lactic dehydrogenase, acid phosphatase, and cathepsin D) were not altered significantly. These results suggest that creatine preferentially stimulates the synthesis of cardiac muscle-specific proteins in vitro and support the hypothesis that creatine may play a role in the development of cardiac hypertrophy.

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