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EFFECT OF A SHORT-TERM DIETARY CREATINE SUPPLEMENTATION ON HIGH-ENERGY PHOSPHATES IN THE RAT MYOCARDIUM

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The aim of this study was to find out whether creatine (Cr) feeding affects total creatine (TCr), phosphocreatine (PCr), adenine nucleotide contents and β-hydroxy-acyl-CoA-dehydrogenase (HAD) activity in myocardium as compared to red skeletal muscle. Ten adult Wistar rats received Cr (2.5 % of diet weight) for 7 days. In Cr fed rats, PCr was increased (by approx. 20%) in cardiac and in soleus muscles with ATP elevated in myocardium and TCr and free Cr in soleus. In both muscles, Cr feeding enhanced HAD activity. It is concluded, that dietary Cr does increase cardiac muscle high energy phosphate reserves and its oxidative potential.

Key words: creatine, phosphocreatine, ATP, β-hydroxy-acyl-CoA-dehydrogenase, rat myocardium, soleus muscle

INTRODUCTION

Dietary creatine (Cr) supplementation for a few days was documented to improve performance of high-intensity exercise in men (1—3). This is attributed to increased ATP resynthesis in working muscles due to enhanced phosphocreatine (PCr) availability in type II fibers. Administration of Cr for 5 days was reported to increase resting PCr content both in type I and and type II fibers (by approx. 15%) in vastus lateralis muscle of healthy subjects (3). An increase in total Cr (TCr) and PCr content in skeletal muscles was also found in Cr fed rats (4—6). However, little is known on the effect of Cr supplementation on the heart. In patients with chronic heart failure Cr ingestion did not improve cardiac performance, evaluated on the basis of ejection fraction, although
exercise tolerance, TCr and PCr in skeletal muscle were enhanced (7). In the only experimental study on the effect of dietary Cr supplementation on the heart in animals, Horn et al. (8) failed to reveal any changes in the rat heart mechanical function, total Cr and PCr or ATP contents despite elevation in serum concentration of Cr by 73 to 202%, depending on the Cr food content.

The influence of muscle PCr content on oxidative potential of myocytes is still unclear. It was reported that depletion of PCr, by means of β-guanidinopropionic acid (GPA) administration, increases activities of mitochondrial enzymes in white (type IIb) skeletal muscle fibers but not in red fibers (type I) or in cardiac muscle (9). The data on the effect of dietary Cr supplementation on skeletal muscle oxidative enzymes are controversial (4,5), and these enzymes were not determined in the heart of Cr fed animals.

The aim of the present work was to investigate the effect of Cr supplementation on the contents of TCr, PCr, adenine nucleotides (ATP, ADP and AMP), as well as on the activity of β-hydroxy-acyl-CoA-dehydrogenase (HAD) in cardiac muscle of the rat. For comparison, the same variables were determined in the red skeletal muscle (soleus).

MATERIALS AND METHODS

The experiments were performed on 20 male Wistar rats, weighing 210 ± 5 g. All animals were housed in temperature controlled quarters (22°C) and had free access to drinking water. The rats were randomly assigned to one of two groups: Ten of them received for seven days 500 mg of monohydrate creatine daily (Now Foods, Glendale Hts., IL, USA) in 20 g of dry rat chow. Powdered chow diet was made to 2.5 % Cr, mixed into a paste with water, formed into pellets, and dried. The control group (10 rats) received daily 20 g of dry (similarly prepared) rat chow without Cr. On the 8th day rats were anesthetized with pentobarbital sodium (60 mg/kg body wt.). The soleus muscle and the apex part of heart ventricles were excised, deep frozen within 15 s in liquid nitrogen, and then stored at −80°C until assayed. The muscle specimens (about 50 mg of each) for determination of PCr, Cr, ATP, ADP and AMP were freeze-dried, dissected free of blood and connective tissue, powdered, and then extracted with perchloric acid. The neutralized extracts were analyzed enzymatically (10). Activity of HAD was determined in wet samples of soleus and cardiac muscles. The reactions catalyzed by this enzyme were coupled to NAD — NADP linked reactions according to Lowry and Passonneau (11).

Energy charge potential (ECP) was calculated according to Atkinson (12). All values are expressed as means with standard errors (SE). Statistical significance was assessed with unpaired Student’s t test. Levels of significance were set at P ≤ 0.05.

RESULTS

Creatine supplementation caused a significant increase of PCr both in cardiac and soleus muscles, by approx. 19% and 21%, respectively, whilst free Cr and TCr contents were enhanced significantly only in the soleus (Table I).
In Cr fed rats, the cardiac muscle content of ATP was significantly enhanced, AMP was diminished, and ADP was unchanged. In the soleus muscle there was only a tendency towards an increase in ATP and a decrease in AMP in comparison with the control group. Neither in the heart nor in the soleus ECP was significantly affected by Cr supplementation.

Table 1. Total creatine (TCr), phosphocreatine (PCr), free creatine (Cr), adenine nucleotides (ATP, ADP, AMP) in μmol·g⁻¹ d.w., energy charge potential (ECP), and PCr to ATP ratio in myocardium and soleus muscle of control (N) and creatine fed (C) rats.

<table>
<thead>
<tr>
<th></th>
<th>TCr</th>
<th>PCr</th>
<th>Cr</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>ECP</th>
<th>PCr/ATP</th>
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</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td></td>
<td></td>
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<tr>
<td>muscle</td>
<td>N</td>
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<td></td>
</tr>
<tr>
<td>C</td>
<td>90.7</td>
<td>38.5</td>
<td>52.2</td>
<td>23.3</td>
<td>4.1</td>
<td>0.37</td>
<td>0.91</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>±2.6</td>
<td>±2.1</td>
<td>±3.2</td>
<td>±1.1</td>
<td>±0.8</td>
<td>±0.06</td>
<td>±0.02</td>
<td>±0.10</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>98.8</td>
<td>45.8*</td>
<td>53.5</td>
<td>30.6*</td>
<td>4.0</td>
<td>0.22*</td>
<td>0.93</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>±3.5</td>
<td>±2.5</td>
<td>±2.6</td>
<td>±2.7</td>
<td>±0.4</td>
<td>±0.07</td>
<td>±0.01</td>
<td>±0.10</td>
</tr>
</tbody>
</table>

Values are means±SE. Asterisks denote significant differences between control and creatine fed rats: *p<0.05, **p<0.01, ***p<0.001.

In control rats, activity of HAD was nearly identical in myocardium and soleus muscle (20.2±1.8 and 20.1±1.3 μmol·min⁻¹ g⁻¹, respectively, and Cr feeding increased this enzyme activity to 34.2±3.9 μmol min⁻¹ g⁻¹ in the heart (p<0.01) and to 29.8±2.8 μmol min⁻¹ g⁻¹ in the soleus (p<0.01).

**DISCUSSION**

The present study showed that in the rat, seven day supplementation with Cr causes similar increases in PCr in cardiac muscle and in the red skeletal muscle, elevating significantly ATP content only in myocardium. This indicates that Cr feeding does enhance cardiac muscle energy reserves. The data did not, therefore, confirm the recent study by Horn et al. (8) who did not find any changes in the high energy phosphate content in myocardium of rats fed various doses of Cr (from 1 to 7% of a diet) for 40 days. The discrepancy between the results obtained by Horn et al. (8) and the present data may be related to duration of Cr feeding. It can be speculated that prolonged increase of extracellular Cr concentration may down-regulate the tissue creatine transporters (13, 14). There are also discrepancies in the literature concerning an influence of Cr supplementation on skeletal muscle PCr content. In the quoted
above study by Horn et al. (8) no effect of Cr feeding on skeletal muscle content of Cr and PCr was found, which is in contrast with the data obtained in human subjects (1, 2, 3) and in rats (4, 5, 6). It should be noted, that in the animal studies duration of Cr feeding and doses of Cr used as well as age and weight of animals varied. Op 't Eijnde et al. (6) who fed rats a high dose of Cr (5 mg g⁻¹ body mass daily) for five days reported an increase in the soleus PCr content by 10 to 20%, which is similar to that found in the present study. Brannon et al. (5) followed up the time-course of changes in PCr and TCr contents in soleus and plantaris muscles up to 24 days after starting Cr feeding at a low dose (0.33% of diet). Their results showed that in both muscles PCr and TCr increased with the most pronounced changes occurring within the first 14 days. Both in the study by Horn et al. (8) and by Tanaka et al. (4) Cr was administered for a long time (40 and 54 days, respectively). However, in the latter study, in which a significant increase in muscle PCr was reported, Cr started to be given to newly weaned rats, while in the former investigation adult animals were used, and their body mass at the end of the experiment was above 400 g. It can be assumed, that ability to transport Cr to the muscle cells is greater in younger than in elder animals.

The important finding of the present study is that concurrently with the enhancement of high energy phosphate content induced by Cr supplementation there was an increase in HAD activity in both myocardium and soleus muscle. This is in line with the results of Brannon et al. (5) who reported an increase in citrate synthase activity in the soleus of Cr fed rats, thus suggesting, that in red skeletal muscles and myocardium an increase in CrP content increases mitochondrial oxidative potential. The data differ from those obtained by Tanaka et al. (4) demonstrating a decrease in HAD activity in the soleus muscle of rats after prolonged supplementation with Cr.

Both the human (15, 16) and animal studies (17) demonstrated that myocardial energy reserves are substantially reduced in chronic heart failure. Thus, the ability of dietary creatine to increase myocardial high energy phosphate content and oxidative potential may be of interest from the clinical point of view, although the role of energy flux by creatine kinase system in maintaining the contractile performance of the heart is still uncertain (17, 18).

REFERENCES


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