JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 105–109

Received May 18, 2001
Accepted August 3, 2001
ISSN 0022-3573

The effects of creatine on the retrogradely perfused isolated rat heart

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Abstract

Although the role of creatine in muscle metabolism is well understood, there is still uncertainty as to its effects at supplemented levels. With this in mind, this study was designed to investigate the direct effects of commercially available creatine on the isolated rat heart, retrogradely perfused and infused with varying concentrations of creatine (1.25, 2.5, 5 and 10 mM) to determine its effects on heart rate, coronary flow and ventricular pressure. Furthermore, tissue from these hearts was used to investigate the cardiotoxic potential of supplemented levels of creatine. Results indicate that creatine directly improves the functioning of the heart under normal conditions with respect to heart rate and ventricular pressure, but may be detrimental to the functioning of energy-deprived hearts. It also showed no cardiotoxic properties since it increased the baseline levels of adenosine triphosphate (ATP) and decreased the levels of isocitrate dehydrogenase (ICD), indicating a decrease in cellular death compared with non-supplemented control hearts.

Introduction

Since the discovery of creatine in 1832, it has fascinated scientists with its role in skeletal muscle metabolism, acting as a catalyst in the transphosphorylation of creatine phosphate to adenosine diphosphate (ADP) to regenerate adenosine triphosphate (ATP) (Walker 1979). Therapeutic uses of creatine supplementation include the treatment of metabolic disorders such as guanidinoacetate methyltransferase deficiency in newborns (Stockler & Hanefeld 1996), while its cyclic analogues, cyclocreatine and homocyclocreatine, have been shown to exhibit anticancer activity (Martin et al 1994). Since the early 1990s, when oral creatine supplements were first made widely available over the counter, it has been surrounded by renewed interest, stemming from our need to attain an ideal body.

Natural sources of creatine include red meat and fish, the typical diet providing approximately 1–2 g of creatine per day (Graham & Hatton 1999). The standard regime for supplementation amounts to a daily intake of 20 g creatine per day. Supplementing with such high doses of creatine has been shown to improve athletic performance by increasing the uptake of creatine by muscle thus making greater quantities of phosphocreatine available for ATP regeneration (Maughan 1995). This has made creatine one of the most popular body supplements despite the lack of scientific research, especially with regards to its side effects (Derman & Schwellnus 1998).

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While the role of supplemented creatine in muscle metabolism and exercise is well understood, its effects on other organ systems is not. Supplementing daily creatine intake with 20 g has been shown to increase urinary creatine excretion from < 150 mg daily to as much as 13 g daily (Poortmans et al 1997; Ropero-Miller et al 2000) and has been shown to result in renal dysfunction in susceptible individuals (Pritchard & Kalra 1998).

With little information available on the short-term, high-dose exposure of creatine on the heart, this study was thus aimed at providing insight into the effects of creatine on cardiac performance. The degree to which cardiac function was increased was measured using heart rate, coronary flow and ventricular pressure as parameters, while also determining the degree to which myocardial ATP content was altered. Isocitrate dehydrogenase (ICD) levels were also determined, indicating the extent of cell damage, as a marker for toxicity.

Materials and Methods

The isolated rat heart model was used to study the direct effect of an infused creatine solution on the heart's function, retrogradely perfused via a modification of the Langendorff method (Langendorff 1895). Thereafter, the same hearts were frozen at −80°C and used to determine the cardiotoxic potential using ATP and ICD levels as markers (Constantin-Teodosia et al 1995).

Perfusion system

The Langendorff perfusion system as described by Lubbe et al (1978) was modified to enable dual perfusion of physiological buffers to alter perfusion conditions. Perfusion pressure was maintained at 1 m of water pressure by means of a Watson–Marlow multi-head peristaltic pump, and the perfusion medium aerated with carbogen (95% O_2, 5% CO_2) (Afrox Pty (Ltd)). Perfusion medium consisted of a modified Krebs–Henseleit physiological buffer (KHB) comprising NaCl (118 mM) (Merck, SA), KH_2PO_4 (1.2 mM) (Merck, SA), $MgSO_4 \cdot 7H_2O$ (1.2 mM) (Saarchem, SA), $CaCl_2 \cdot 2H_2O$ (2.5 mM), $NaHCO_3$ (25 mM) (Merck, SA) and KCl (5.9 mM) (Merck, SA) with glucose monohydrate (11 mM) (Merck, SA) as substrate. The temperature of the perfusion medium was maintained at 37°C. Solutions of creatine were infused by means of a Harvard Apparatus (Model 55-2219) syringe driver infusion pump (Harvard Apparatus, MA) directly into the bubble trap of the perfusion glassware.

Anaesthesia, dissection and mounting

Male Long Evans rats (250–350 g) were anaesthetised under ether (Merck, SA) in a desiccator to a loss of blink and pain reflexes. To prevent formation of blood clots in the coronary vessels, 200 IU of heparin sodium (Intramed, SA) was injected into the femoral vein. Hearts were removed and arrested by immersion in a solution of ice-cold KHB and mounted on the perfusion apparatus by positioning the aorta over an aortic cannula. A platinum ECG electrode was inserted into the myocardium of the right ventricle. A stabilization period of 15 min was allowed before the commencement of the experiment.

Effects on heart rate

After a 15-min stabilization period, a 50 mM creatine solution, made up in milliQ water, was infused at a rate of 0.25 mL min^{-1} for every 10 mL min^{-1} of coronary flow, so that the heart was exposed to an eventual concentration of 1.25 mM creatine. This concentration was based on a study by Schedel et al (1999) which showed peak creatine levels to be an average of 2.17 mM 2.5 h after the ingestion of 20 g of creatine powder, the average loading dose recommended by manufacturers.

An ECG was recorded using an ECG amplifier (Microplex Electronics), Phillips PM3350A storage oscilloscope (Phillips electronics) and Polyview data acquisition software (Grass Instruments). Heart rates were derived from the ECG and recorded at 5-min intervals, along with coronary flow rate (mL min^{-1}).

Effects on ventricular pressure

Separate experiments were performed to determine the effects of creatine on ventricular pressure. Rat hearts were mounted in the same manner as described above, but with a PVC balloon attached to a pressure transducer (Grass Instruments) inserted into the left ventricle through the bicuspid valve, made possible by removal of the left atrium and inflated to a diastolic pressure of 10 mmHg. Hearts were all paced at 340 beats min^{-1} by means of a Grass SIV5 Stimulus isolator unit (Grass Instruments) by placing the electrodes of the unit in contact with the myocardium of the left ventricle.

Varying concentrations of creatine were used. Initially, infusion of a 100 mM solution occurred for a period of 10 min at a rate of 0.25 mL min^{-1} for every 10 mL min^{-1} of coronary flow, after which the infusion rate was doubled at 10-min intervals to 0.5 mL min^{-1} and 1 mL min^{-1} to obtain concentrations of 2.5, 5 and 10 mM creatine, respectively. After 30 min, the perfusion

buffer was changed to a glucose-free KHB to determine the effects of high doses of creatine on lactate and free-fatty-acid metabolism in carbohydrate-deficient tissue. Exposure of the hearts to glucose-free KHB occurred for a period of 10 min, after which the hearts were frozen at −80°C until analysis.

Preparation of cardiac tissue for ATP and ICD assays

All procedures were carried out at 4°C. Samples of tissue were thawed on ice, sliced, weighed and homogenized in 10 volumes of 20 mM Bis-Tris (Sigma, USA), 5 mM 2-mercaptoethanol (Sigma, USA), 2 mM benzamidine (Sigma, USA), 2 mM EDTA (Merck, SA) and 50 mM sodium acetate (Merck, SA) (Reeves et al 1987). To block ATPase activity, phenylmethanesulphonyl fluoride (Sigma, USA) was dissolved in propan-2-ol (Merck, SA) and added to the buffer immediately before homogenization to give a final concentration of 50 μM (Reeves et al 1987). The homogenate was centrifuged at room temperature for 5 min at 3500 rev min^{-1} and then for 30 mins at 10000 rev min^{-1}. The supernatant was removed and utilized in the determination of ATP and ICD concentrations.

Determination of myocardial ATP and ICD concentrations

ATP and ICD levels were determined using the ATP Assay Kit (Sigma) and ICD Assay Kit (Sigma) respectively, according to manufacturer's specifications.

Statistical methods

Results were reflected as means±s.d. and graphed as a function of time; significant differences in means were determined using a paired *t*-test performed with the aid of Prism 3 software (Graphpad). Significance was defined as a *P* value of less than 0.01.

Results and Discussion

Effects of creatine on heart rate

A decrease in heart rate for the control experiments was noted (Figure 1), which is expected as conditions for isolated organs are not ideal. However, those hearts infused with 1.25 mM creatine showed resiliency towards this natural decrease in heart rate, with the average heart rate after 30 min of creatine infusion being significantly higher than the mean heart rate before infusion. This phenomenon is likely to occur due to creatine's action in increasing ATP levels in muscle tissue (Guervero-Ontiveros & Wallimann 1998; Kyne 1999). The mean heart rate after creatine infusion for 30 min was significantly greater than that of the control hearts ($P < 0.001$), showing an improvement in cardiac function with respect to heart rate. However, these results were achieved using hearts obtained from healthy male rats, thus not excluding contra-indicated use of creatine in those individuals suffering from any cardiac abnormalities.

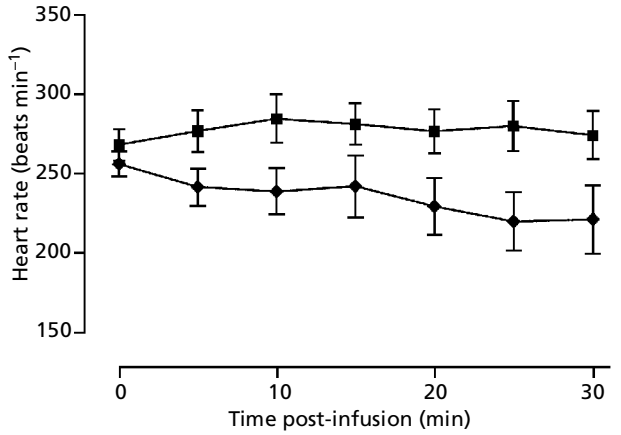

Figure 1 Effects of creatine 1.25 mM on heart rate of isolated perfused rat hearts (■, n = 8) compared with control group (◆, n = 8). Points represent means±s.d., $P = 0.0004$, compared with control (all means).

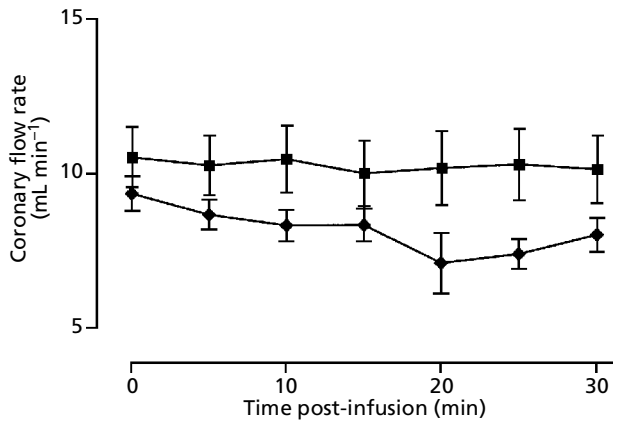

Figure 2 Effects of creatine 1.25 mM on coronary flow in isolated perfused rat hearts (■, n = 8) compared with control group (◆, n = 8). Points represent means±s.d., $P = 0.0002$, compared with control (all means).

The effects of 1.25 mM creatine infusion on coronary flow (Figure 2) was also observed during heart-rate experiments, showing the coronary flow in creatine-infused heart to be significantly higher than that in the

control group hearts ($P < 0.001$). This also showed an improvement in cardiac function. This phenomenon may be explained by the increase in heart rate resulting in an improvement in perfusion of coronary vasculature. An improvement in coronary blood supply may be beneficial during strenuous exercise, which is commonly associated with supplementation of large amounts of creatine (Balsom et al 1994).

Effects of creatine on ventricular pressure

Results of experiments testing the effects of creatine on left ventricular systolic pressure (LVSP) (Figure 3) followed the same trend as those for heart rate, with mean LVSP being higher than that for the control experiments ($P < 0.001$). In this series, varying concentrations of creatine were used (2.5, 5, and 10 mM), showing no significant dose–response relationship. At all concentrations, LVSP was higher than that of the control group with mean differences between controls and experiments not being significantly different for increasing concentration of creatine. This may be due to saturation of effects once certain concentrations are reached, signifying that large increases in supplemented doses may have no additional cardiac benefits, while increased detrimental effects on other organ systems may not be excluded. No conduction abnormalities were noted in the first 30 min of infusion. However, after perfusion medium was switched to glucose-free KHB, increases in atopic beats and ventricular tachycardia and arrhythmia were noted more frequently in hearts infused with creatine than those without (60% occurrence of conduction abnormalities in creatine-infused hearts vs 25% occurrence in control hearts). This may pose a significant clinical problem for individuals using high doses of creatine supplements who may have decreased cardiac glucose supplies. This phenomenon may also be explained by the augmented uptake of creatine into muscle cells by the simultaneous administration of large amounts of carbohydrates (Green et al 1996), thus reducing the uptake of creatine into cardiac cells that have relied on the creatine supplementation to maintain a faster rate and more forceful contractions, thus leading to the increased likelihood of conduction abnormalities.

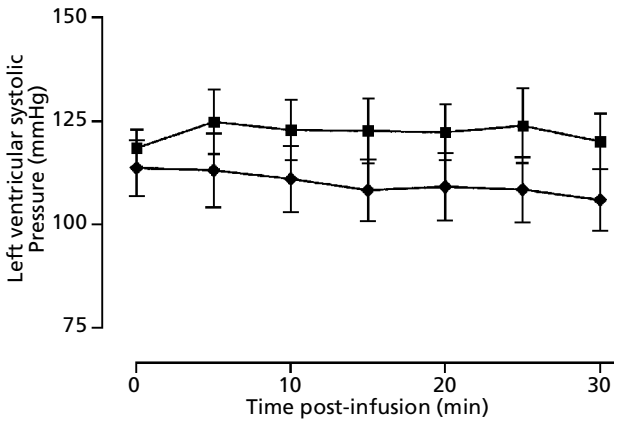

Figure 3 Left ventricular systolic pressure (LVSP) in isolated rat hearts vs time. Experimental group (■, n = 8); control group (◆, n = 8). Creatine 2.5 mM was infused at time 0 min; the concentration was doubled at 10 min and again at 20 min, to give a final concentration of 10 mM. No correlation existed between creatine concentration and LVSP.

ATP assay

Infusion of supplemented levels of creatine was shown to significantly increase myocardial ATP concentrations

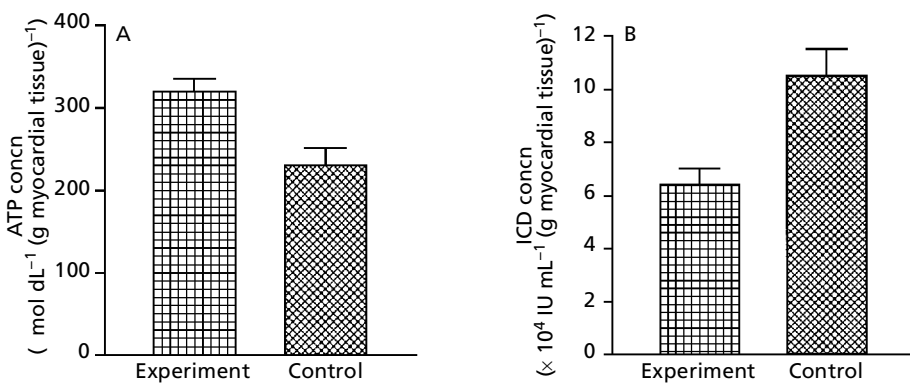

Figure 4 ATP (A) and isocitrate dehydrogenase (ICD) (B) concentrations of myocardial tissue exposed to creatine in left ventricular systolic pressure (LVSP) experiments (isolated rat heart was perfused with 2.5 mM creatine at time 0; this was doubled at 10 min and again at 20 min to give a final concentration of 10 mM). Tissue exposed to supplemental levels of creatine showed significantly higher levels of ATP than the control group ($P = 0.0087$) but showed lower levels of ICD than the control ($P = 0.0085$).

(P = 0.0087) (Figure 4A). This result was expected due to the metabolic actions of creatine in muscle tissue. An increase in ATP content of hearts exposed to supplemented levels of creatine also explains the increase in heart rate and ventricular pressure noted in the isolated heart experiments since increased ATP production results in higher levels of cAMP, and hence promotes the mobilization of calcium (Walker 1979).

ICD assay

Creatine-exposed hearts showed a significant decrease in ICD concentration as compared with the control group (P = 0.0085) (Figure 4B). The ICD content of myocardial tissue is directly proportional to the extent of cell death (Devlin 1992), showing a decrease in cellular death for myocardial tissue exposed to supplemented levels of creatine. Creatine supplementation thus proves to have a beneficial cardioprotective action. This, however, is only the case for short-term supplementation of creatine and does not preclude the possibility of adverse cardiac effects in long-term, high-dose supplementation.

Conclusion

It can thus be concluded that short-term exposure to high levels of creatine (similar to those expected in individuals who use creatine as a sport supplement) have no significant adverse effects in retrogradely perfused isolated rat hearts. Effects on heart rate, coronary flow and ventricular pressure have been shown to be beneficial, improving total cardiac function as well as decreasing cellular injury and death and increasing available myocardial ATP. It must be emphasized that these results are an indication of the cardiac effects of individuals using the supplement on a short-term basis and can not exclude the possibility of adverse effects of long-term supplementation with large doses of creatine. It must also be noted that beneficial effects were only noted for optimal perfusion conditions, but when glucose supply was limited, conduction abnormalities occurred at a greater frequency in creatine-supplemented hearts as compared with the control group. Therefore, it is not advised that individuals suffering from any coronary artery disease or abnormality should supplement with high doses of creatine.

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