Actions of the creatine analogue β -guanidinopropionic acid on rat heart mitochondria

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The action of the creatine analogue β -guanidinopropionic acid (β -GPA) was examined in rat heart mitochondria and in isolated cardiomyocytes or fibres which were permeabilized with the non-ionic detergent saponin to determine the kinetics of mitochondrial creatine kinase for β -GPA. Fibres and myocytes were subjected to increasing [ADP] in the presence and absence of β -GPA or creatine, whereas isolated mitochondria received a similar protocol with increasing [ATP]. In isolated mitochondria given ATP, there was a stimulation of respiration by creatine, but no significant stimulation of respiration by β -GPA. Further studies on fibres from control and β -GPA-fed rats also found that β -GPA is not utilized by the mitochondria, as evidenced by a lack

of β -GPA-stimulated respiration (K_m for ADP = $142 \pm 23 \mu\text{M}$) compared with control (K_m for ADP from $161 \pm 23 \mu\text{M}$), but no significant change in V_{max} . Therefore the rat heart mitochondria are not responsive to β -GPA as compared with creatine. Interestingly, the fibres from β -GPA-fed rats had no creatine- or β -GPA-stimulated respiration (K_m for ADP = $57.3 \pm 7.2 \mu\text{M}$ for control, $54.2 \pm 7.2 \mu\text{M}$ with creatine, and $53.5 \pm 7.8 \mu\text{M}$ with β -GPA). The mitochondria prepared from the hearts of rats exposed for 10 weeks to 1% β -GPA in their diet had a significant decrease in V_{max} and a significant decrease in K_m for ADP. Thus the hearts from β -GPA-fed animals may be pathologic, due to a disruption of the creatine kinase energy circuit.

INTRODUCTION

Experiments using β -guanidinopropionic acid (β -GPA) as a creatine analogue have been performed with the aim of understanding the role of the creatine kinase (CK) system in the cell. These experiments are based on the assumption that β -GPA is used by the CK system essentially with the same kinetics *in vivo* as *in vitro*. There is, however, a growing body of evidence that the behaviour of an enzyme in solution, and that seen *in vivo* or *in situ*, are not identical when one compares observations in solution and in the tissue (Dillon and Clark, 1990; Srivastava and Bernhard, 1986; Crabtree and Newsholme, 1978). *In vitro*, the K_m of CK for β -GPA is twice that for creatine, with a V_{max} that is 3 orders of magnitude less than for phosphocreatine (PCr) (Chevli and Fitch, 1979). If such kinetics also held in the tissue, then it would be unlikely that phosphorylation of β -GPA and hydrolysis of its phosphate (β -GPAP) would occur acutely. However, relatively rapid phosphorylation of β -GPA in the isolated perfused rat heart (Unitt et al., 1993) and hydrolysis of β -GPAP (the phosphorylated form of β -GPA) has been observed (Roberts and Walker, 1982), inferring that the properties of CK *in vitro* are different from those *in vivo*.

Recent experiments with β -GPA feeding have shown the development of mitochondrial paracrystalline inclusions in these animals (Eppenberger-Eberhardt et al., 1991; Wallimann et al., 1992; Wyss et al., 1992). These inclusions have been identified as consisting largely of mitochondrial CK (Mi-CK). Eppenberger-Eberhardt et al. (1991) found similar inclusions in the mitochondria of cultured cardiomyocytes grown in the absence of creatine. They attributed the development of the crystals to a proliferation of Mi-CK in response to a lowered energy state in

the cells. Another possibility, however, is that the Mi-CK cannot act on or respond to β -GPA as it does to creatine, and it may therefore be a low [creatine] at the Mi-CK (and not the energy state of the cell) that is the signal for Mi-CK proliferation. Zweier et al. (1991) reported no stimulation of respiration from β -GPA using isolated rat heart mitochondria, even with concentrations of up to 200 mM. β -GPA did, however, exhibit weak competitive inhibition, with a K_i of $94 \mu\text{M}$. It is apparent from these data that the kinetic behaviour of β -GPA is different from that of creatine in its effect on isolated mitochondria, bringing into question the ability of Mi-CK to phosphorylate β -GPA.

This study was undertaken to determine if there is a metabolic response of the mitochondrial CK system to the creatine analogue β -GPA. Isolated mitochondria, cardiomyocytes and skinned heart muscle fibres from normal rats and β -GPA-fed rats (fed on 1% β -GPA for 10 weeks) were studied, and the results suggest that the rat heart mitochondrial Mi-CK is insensitive to the creatine analogue β -GPA.

METHODS

Heart preparation

Rats were anaesthetized with an intraperitoneal injection of 1 ml of pentobarbital/kg body wt. Hearts were rapidly removed and placed in cold (4 °C) Krebs buffer (Field et al., 1994).

Isolation of mitochondria

Rat heart mitochondria were isolated by a trypsin digestion procedure as described by Saks et al. (1985). Hearts were finely minced with surgical scissors in a solution of 0.3 M sucrose,

Abbreviations used: PCr, phosphocreatine; β -GPA, β -guanidinopropionic acid; β -GPAP, β -guanidinopropionic acid phosphate; CK, creatine kinase; Mi-CK, mitochondrial CK.

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10 mM Na-Hepes and 0.2 mM EDTA, pH 7.2 at 4 °C. The mince was incubated for 15 min with 125 mg/ml trypsin (Fluka), followed by gentle homogenization. The homogenate was centrifuged at 1000 *g* for 10 min and the pellet discarded. The supernatant was centrifuged at 8000 *g* for 15 min. The mitochondria in the resulting pellet were resuspended in isolation medium containing 1.0 mg/ml BSA and the centrifugation was repeated.

Cardiomyocyte preparation

Fresh myocytes were prepared by the technique described by Saks et al. (1991, 1993). Rats were anaesthetized and the heart was excised quickly and placed in ice-cold Krebs buffer containing (mM) 117 NaCl, 5.7 KCl, 4.4 NaHCO₃, 1.5 KH₂PO₄, 1.7 MgCl₂ at pH 7.1. The hearts were cannulated and perfused at a flow rate of 6 ml/min during a 5 min Ca²⁺-free perfusion; 1.25 mg/ml collagenase (Sigma) was then added, and the perfusion continued for 1 h. Perfusion with 50 ml of collagenase solution was performed in a recirculating system continuously aerated at 37 °C. After the perfusion, the heart was removed and ruptured mechanically by forceps to release the cardiomyocytes. Suspended cells were filtered to separate them from the bulk myocardial mass and transferred to test tubes for 5 min for gravity sedimentation. The cardiomyocyte pellet was resuspended in a solution containing 20 µM Ca²⁺ and 5 mg/ml fatty-acid-free BSA and washed again. The final suspension of myocytes was prepared in the respiration solution (R) for addition into the oxygen-consumption chamber.

Skinned-fibre or myocyte respiration experiments

Mitochondrial respiratory parameters in saponin-skinned fibres and myocytes were determined by the method described previously for heart muscle (Veksler et al., 1987). By using saponin (40 µg/ml), the plasma membrane was selectively permeabilized while leaving membranes of the mitochondria and sarcoplasmic reticulum intact and functional.

Fibre preparation

Fibre bundles of diameter approx. 200 µm were isolated from the left ventricle of freshly excised rat heart and transferred into skinning solutions (S, see below). Bundles were incubated with intense stirring for 20 min in solution S containing 40 µg/ml saponin (Calbiochem) (which leaves mitochondrial function intact). Bundles were then washed for 10 min in respiration solution (R, see below) without high-energy phosphates at 4 °C.

Measurement of respiratory parameters

The respiratory rates were determined by a Clark oxygen electrode (Hanasatech) in an oxygraph cell containing a mitochondrial suspension, myocytes, or 2–5 mg dry wt. of fibres, in solution R at 30 °C with continuous stirring. The solubility of oxygen was taken to be 400 ng-atoms of O/ml. Fibres, myocytes or mitochondria were introduced into the oxygraph cell, and oxygen consumption was measured during sequential substrate addition. Fibres were then dried and weighed. Respiration rates were expressed as ng-atoms of O/min per mg dry wt. Myocytes and mitochondria were assayed for protein content by the Bio-Rad protein assay, and results are reported as ng-atoms of O/min per mg of protein.

Kinetics

ADP kinetic experiments were performed on isolated myocytes and rat heart fibres. Fibres or myocytes were introduced to the oxygraph cell and exposed to increasing [ADP] in the presence of creatine or β-GPA (25 mM). The ADP-stimulated respiration above basal oxygen consumption was plotted in order to determine the apparent *K_m* for ADP and *V_{max}*. Other experiments on mitochondria were done by addition of ATP to determine the apparent *K_m* for the mitochondria to ATP in the presence of creatine or β-GPA.

Solutions

Skinning solution (S) and respiration solution (R) contained (mM) EGTA 10 (pCa 7.0), free Mg²⁺ 3, taurine 20, dithiothreitol 0.5 and imidazole 20 (pH 7.0). Free Ca²⁺ and Mg²⁺ concentrations were calculated on the basis of the equations described by Fabiato and Fabiato (1979), by utilizing the values of dissociation constants. Ionic strength was adjusted to 0.16 M by addition of K-Mes. Solution S also contained 5 mM MgATP and 15 mM PCr. In place of high-energy phosphates, solution R contained 5 mM glutamate, 2 mM malate, 3 mM phosphate and 2 mg/ml fatty-acid-free BSA.

β-GPA administration

Weanling Wistar rats were fed on regular laboratory chow supplemented with 1% (w/w) β-GPA for 10 weeks with free access to food and water. Age-matched rats were similarly housed and treated while being fed regular laboratory chow without β-GPA.

N.m.r. methods

N.m.r. experiments were performed on isolated perfused rat hearts to demonstrate the amount of PCr substitution after 10 weeks of chronic β-GPA feeding. Hearts were perfused in the Langendorff mode with a Krebs buffer solution. By using 128 transients collected with a 4 s relaxation delay and Fourier-transformed with 15 Hz line broadening, heart spectra showed β-GPAP accumulation. All ³¹P-n.m.r. experiments were performed on a Bruker Biospec 3000 instrument at 161.9 MHz.

Materials

All chemicals were purchased from Sigma unless stated otherwise.

Statistics

The test for significance was Student's *t* test. Results are reported as means ± S.E.M.

RESULTS

Mitochondria

Figure 1 show a double-reciprocal plot of 1/oxygen consumption (in ng-atoms of O/min per mg dry wt.) versus 1/[ATP] for mitochondrial respiration experiments in the presence or absence (control) of β-GPA or creatine; *V_{max}* is unaffected by β-GPA. Addition of creatine did, however, significantly decrease the apparent *K_m* of the mitochondrial respiration for ATP. This is consistent with results reported by Saks et al. (1991), which was termed creatine-stimulated respiration (Jacobus and Saks, 1982; Jacobus, 1985). β-GPA had no significant effect on the mito-

Figure 1 Double-reciprocal plot of representative responses from isolated mitochondria to [ATP]

To the medium was added 25 mM creatine or 25 mM β -GPA. ATP concentration is in μ M.

Table 1 Respiration by isolated mitochondria

* Significantly different from control ($P < 0.05$). n is the number of kinetic experiments performed on five mitochondrial preparations

	$V_{\max}$ (ng-atoms of O/min per mg of protein)	K_m (μ M ATP)	n
Control	273.7 ± 15.5	46.9 ± 3.57	10
Creatine	$618.8 \pm 51.3^*$	$23.4 \pm 2.95^*$	6
β -GPA	280.7 ± 24.5	58.8 ± 4.76	9

chondrial response to ATP compared with control (see Table 1). We conclude that there was no β -GPA-stimulated respiration.

The stimulation of respiration by ATP showed that, in the presence of creatine, there is a lower apparent K_m for ATP with a significant increase in $V_{\max}$. In the control experiments, with ATP alone, the respiration is supported by ADP production in the MgATPase reaction (mitochondrial or microsomal in origin), and the kinetic parameters reported are those characterizing this reaction in the mitochondrial preparation. In the presence of creatine, the CK reaction is an additional source of ADP; that explains the elevated $V_{\max}$. The decreased K_m value reflects the effective coupling between CK and oxidative phosphorylation (Saks et al., 1991; Jacobus and Saks, 1982). The apparent K_m and $V_{\max}$ for the isolated mitochondria, in the presence of β -GPA, were not significantly different from control. This supports the observation that β -GPA was not phosphorylated by the mitochondrial CK, and so ADP was therefore not being produced by the ATPase activity. Furthermore, β -GPA did not inhibit the mitochondria, because there was no difference in the responses to ATP (Figure 1). This is in contrast with creatine, which was phosphorylated, producing PCr and ADP, with concomitant stimulation of respiration as well as a decreased apparent K_m for ATP.

Myocytes

Figure 2 is a representative oxygen trace in the presence of 25 mM β -GPA. In this experiment 25 mM creatine was added after 0.1 mM ADP (β -GPA present throughout). The addition of creatine produced an increase in the rate of oxygen consumption,

Figure 2 Representative oxygen-consumption trace from skinned rat heart myocytes in the presence of 25 mM β -GPA throughout the experiment

Addition of 25 mM creatine at E produced a characteristic increase in respiration in the presence of 0.1 mM ADP. The creatine-stimulated respiration occurs despite the presence of β -GPA. The vertical and horizontal bars represent 10 ng-atoms of O and 2 min respectively.

Figure 3 (a) Double-reciprocal plot of the creatine-stimulated respiration seen in representative saponin-skinned fibres from the same rat heart; (b) double-reciprocal plot obtained from saponin-permeabilized heart fibres in the presence and absence of 25 mM β -GPA

There is a distinct lack of β -GPA-stimulated respiration, with no significant difference in the apparent K_m in the presence of β -GPA. Oxygen consumption is expressed as ng-atoms of O/min per mg dry wt. Lines are best fit with linear regression. $\square$, Control; $\circ$, in the presence of 25 mM creatine; $\bullet$, in the presence of 25 mM β -GPA.

showing that creatine-stimulated respiration occurred regardless of the presence of β -GPA. Thus β -GPA did not prevent creatine stimulation of mitochondrial respiration, nor did it significantly affect the maximal velocity (results not shown).

Table 2 Anatomical parameters for β -GPA-fed ratsValues are means $\pm$ S.E.M. for n experiments. NS, not significant

	Body wt. (g)	Heart wt. (g wet wt.)	$10^3 \times$ Heart wt./body wt.	H ₂ O (%)	n
Control	391.3 $\pm$ 13.4	1.15 $\pm$ 0.04	2.93 $\pm$ 0.05	74.4 $\pm$ 1.7	4
β -GPA-fed	361.5 $\pm$ 11.1	1.35 $\pm$ 0.19	3.79 $\pm$ 0.10	76.6 $\pm$ 0.36	5
P	NS	0.01	0.001	NS

Figure 4 Representative n.m.r. spectrum from a rat heart after 10 weeks of chronic administration of β -GPA

Peaks: A, frequency marker; B, extracellular P_i ; C, intracellular P_i ; D, phosphocreatine; E, β -GPAP; F, G and H are γ , α and β ATP peaks.

Table 3 Respiration of skinned fibres from β -GPA-fed rats

Results are for a minimum of 9 fibre bundles for each experiment from 4 or 5 hearts, and are means $\pm$ S.E.M. where indicated. 25 mM creatine or β -GPA was included in the respiration solution. K_m for ADP is given in μ M, and V_{max} is in ng-atoms of O/min per mg dry wt. *Significantly less than control K_m . NS, not significant.

	Solution R		R + creatine		R + β -GPA	
	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m
Control	32.2 $\pm$ 5.4	161 $\pm$ 28	24.5 $\pm$ 1.5	64.0 $\pm$ 8.4*	27.1 $\pm$ 5.8	142 $\pm$ 23
β -GPA-fed	11.1 $\pm$ 2.1	57.3 $\pm$ 7.2	11.7 $\pm$ 2.2	54.2 $\pm$ 7.2	11.2 $\pm$ 1.9	53.5 $\pm$ 7.8
P	0.001	0.01	0.01	NS	0.05	0.01

Fibres

Experiments were also performed with saponin-permeabilized myocardial fibres, and creatine-stimulated respiration was observed as a decrease in apparent K_m for ADP (Figure 3a) with no significant difference in the respiratory responses in the presence of β -GPA compared with control (Figure 3b). This similarity between the responses of fibres and cardiomyocytes is consistent with the results of Khuchua et al. (1989, 1992) on creatine-

Figure 5 Double-reciprocal plot for heart fibres from rats given 1% β -GPA for 10 weeks

Creatine or β -GPA (25 mM) produced no significant change in the response, as seen in these representative experiments. Oxygen consumption is expressed as ng-atoms of O/min per mg dry wt. Lines are best fit with linear regression. $\square$, Control; $\circ$, in the presence of 25 mM creatine; $\triangle$, in the presence of 25 mM β -GPA.

stimulated respiration in the myocytes and fibres of the hamster. They concluded that diffusion distances for ADP do not restrict the response to creatine.

Chronic β -GPA administration

After rats were fed for 10 weeks on a diet containing 1% β -GPA, the hearts showed significant hypertrophy (Table 2). There was significant loading of β -GPA, as seen in Figure 4, with the large β -GPAP peak compared with PCr. To evaluate if hypertrophy was an adaptive mechanism involving mitochondrial oxidative phosphorylation, we repeated the kinetic experiments on the cardiac fibres. We showed a significant decrease in the V_{max} and K_m for ADP in heart fibres of β -GPA-fed rats compared with pair-fed controls (Table 3). There was again no significant difference in the kinetic parameters measured between the fibres of β -GPA-fed rats in the presence of β -GPA or creatine (Figure 5). Therefore there was no adaptation of the heart mitochondria enabling them to utilize β -GPA.

DISCUSSION

Creatine-stimulated respiration

The mechanism for creatine-stimulated respiration occurs when Mi-CK is functionally coupled to oxidative phosphorylation. ADP is produced and then enters the mitochondrial matrix via the adenine nucleotide translocase and stimulates oxidative phosphorylation (Saks et al. 1991; Jacobus and Saks, 1982; Bessman and Geiger, 1981). It was our intention to examine the respiratory responses of the mitochondria, and we found that Mi-CK did not use exogenous ATP to phosphorylate β -GPA to a significant extent.

The change in apparent K_m seen in the isolated mitochondria (Figure 1) can be explained by ADP production in a micro-compartment at the inter-mitochondrial membrane space. Nascent ADP produced by Mi-CK is transported into the mitochondria, with concomitant stimulation of respiration. The result is an apparent increase in affinity for ATP caused by the Mi-CK, producing a higher local concentration of ADP. In the experi-

ments presented here we show that myocytes as well as skinned fibres are unable to utilize β -GPA, because there was no β -GPA-stimulated respiration with no observable effect on creatine stimulated respiration.

Mitochondrial kinetics

The presence of β -GPAP in chronically treated heart muscle has been thought to cause a short circuit of the creatine-PCr circuit (Meyer et al., 1986). It was proposed that β -GPA administration produced a characteristic metabolic response to increased work (Mekhfi et al., 1990; Kapelko et al., 1989; Shoubridge et al., 1985). Functional coupling of Mi-CK to oxidative phosphorylation has been shown to affect its kinetic behaviour (Bessman and Geiger, 1981; Saks et al., 1985). Possible causes for the apparent inability of β -GPA to be phosphorylated by Mi-CK are: (1) it may not be able to cross the mitochondrial outer membrane, or some other physical barrier; or (2) Mi-CK specificity.

ATP-stimulated respiration in isolated mitochondria results in mitochondrial ATPases producing ADP. Thus the apparent K_m obtained reflects the affinity of the ATPases and their ADP production. V_{max} in these conditions, however, is limited more by the maximal rate of ADP production than by the maximal rate of oxygen consumption. In our experiments, V_{max} in the presence of β -GPA was not significantly different from control, whereas creatine caused a significant increase in V_{max} . The increase in V_{max} is due to an increased rate of ADP production in the intermembrane space by Mi-CK. Because β -GPA did not affect V_{max} of the isolated mitochondria, we believe that β -GPA was not being phosphorylated to a significant extent.

It is possible that the lack of β -GPA-stimulated respiration is due to the lower affinity of Mi-CK for β -GPA. We found, however, that even in the presence of 100 mM β -GPA respiration was not stimulated (results not shown). If one takes into account the observation that the response of the mitochondria to a low-creatine environment is the proliferation of Mi-CK, and that a similar proliferation is seen with chronic exposure to β -GPA, then it is reasonable to suppose that the mitochondria are responding to the β -GPA treatment as an effective lack of creatine [though a low PCr/ATP or ATP/ADP (altered energy state) may be involved as well]. We believe that the metabolic responsiveness of an enzymic system is affected by the kinetics (K_m and/or V_{max}) of the substrate and that a disruption of this system can lead to pathology such as hypertrophy.

Hypertrophy

Mi-CK has been shown by Wallimann et al. (1992) and Eppenberger-Eberhardt et al. (1991) to proliferate and form paracrystalline structures in the mitochondria of animals fed on β -GPA and in myocytes cultured in the absence of creatine. They concluded that the mitochondria were responding to the energy status of the cell and that a deficiency in PCr was the signal that caused a proliferation of Mi-CK. We believe that the kinetic difference between β -GPA and creatine (Chevli and Fitch, 1979) may be a contributing factor leading to the paracrystalline inclusions.

Zweier et al. (1991) used β -GPA to decrease creatine and PCr in rat hearts and concluded that PCr may function by optimizing the utilization of cardiac high-energy phosphates. Roberts and Walker (1982) found that heart muscle of β -GPA-fed rats hydrolysed ATP with a similar time course to that for control muscle fibres during ischaemia, whereas cyclocreatine-fed rats resisted development of rigor and were able to maintain ATP. β -

GPAP synthesized and accumulated by the rat heart may be utilized as a synthetic phosphagen, because β -GPAP was hydrolysed during ischaemia (Roberts and Walker, 1982; Shoubridge et al., 1985), though subcellular localization was not addressed. β -GPA does not inhibit creatine-stimulated respiration (Figure 2), nor does it stimulate respiration. Our results support the possibility that the mitochondria may be responding to the Mi-CK system lacking creatine (which may be an essential substrate for the mitochondria, and β -GPA is not a substitute).

We wish to propose that the tissue pathology may be due to or exacerbated by mechanisms including (but not limited to) the altered cellular energy status and creatine deficiency. The impaired energy status in the cell may contribute to the pathology of the tissue, while the mitochondrial changes may be affected by the lack of substrate for Mi-CK. If rat cardiomyocytes are incubated in the absence of creatine, proliferation of Mi-CK may result in the formation of paracrystalline inclusions (of Mi-CK) seen by Wallimann et al. (1992) and Eppenberger-Eberhardt et al. (1991).

To investigate whether the pathology associated with β -GPA administration had an effect on the heart mitochondria, we performed respiration experiments on fibres from rats fed on β -GPA for 10 weeks. We found that in the hypertrophied heart fibres there was no observable response to β -GPA. These fibres had a decrease in soluble Mi-CK activity and had no observable response to creatine. There was, however, a significant decrease in V_{max} and a decreased apparent K_m for ADP. The decrease in V_{max} may be explainable by the increase in heart mass seen during hypertrophy and/or pathological changes in the mitochondria. The decrease in apparent K_m , observed in the saponin-skinned fibres, can be explained by an increased affinity for ADP.

Elevated ADP in the heart can lead to an increase in stiffness and thus decrease cardiac function (Veksler et al., 1991; Ventura-Clapier et al., 1988). A decrease in the apparent K_m for ADP by the mitochondria might be a beneficial response to prevent an elevation in [ADP], as well as helping to maintain [ATP], as has been reported previously (Field et al., 1991, 1994).

In the mitochondria, there could be several steps where the apparent K_m might be altered. The observed decrease in apparent K_m may be due to an increased affinity of the F_1 ATP synthase for ADP (and/or an increase in the F_1 ATP synthase) or the adenine nucleotide translocase. The apparent K_m for ADP could be decreased if the amount of non-catalytic collisions decreased. Cardiac hypertrophy increases the F_1 ATP synthase activity (Das and Harris, 1990, 1991, 1993), and this may contribute to the increased affinity for ADP. The above is a simple case, and the mitochondrial system is more complicated, but the potential cause for the altered apparent K_m may be due to an increase in F_1 ATP synthase or translocase.

The hypertrophic heart may have increased diffusion limitations for ADP. We have indeed found diffusion limitations to ADP in rat heart mitochondria (Saks et al., 1991; Khuchua et al., 1992). Though there is evidence that CK binding to contractile proteins does not affect kinetics (Dowell and Fu, 1992), others have found diffusion restrictions with immobilized enzymes (Arrio-Dupont et al., 1992). However, we have no evidence as to whether hypertrophy increases diffusion limitations in the fibres.

The decreased apparent K_m seen in the hypertrophic fibres may indeed be a beneficial adaptation for the fibres. It is noteworthy that, in control rats, there is no significant difference between the apparent K_m for ADP in the presence of creatine and for the hypertrophic heart fibres.

Thus, as the Mi-CK system is becoming less active, the apparent K_m for ADP is nonetheless maintained possibly to maintain ADP and or ATP concentrations.

Conclusions

In this paper we have shown that the mitochondrial CK cannot substitute β -GPA for creatine, and that the hypertrophy caused by chronic administration of β -GPA leads to an increased affinity for ADP by the mitochondria.

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