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MITOCHONDRIAL CREATINE PHOSPHOKINASE DEFICIENCY IN DIABETIC RAT HEART

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The role of the mitochondrial end of the phosphocreatine energy shuttle was studied in the streptozotocin diabetic rat heart. Diabetic rats had 45±5% lower body weight and yielded 46±6% less mitochondria/gm of protein than normals. Diabetic heart mitochondria had 32±7% lower creatine phosphokinase (CPK) activity and 59±10% lower oxygen consumption rate than normal heart mitochondria. Creatine (25 mM) did not stimulate oxygen uptake by diabetic heart although control (normal) heart mitochondria were stimulated. Inadequate mitochondrial energy production in the form of phosphocreatine could result in lower energy delivery to the myofibrillar contraction sites and might be an important factor in diabetic cardiomyopathy and weight loss.

For the last 15 to 20 years data have been presented in the literature to support the existence of a specific diabetic cardiomyopathy independent of atherosclerosis, coronary artery disease and hypertension (1-5). The findings indicate that chronic diabetes diminishes contractility and prolongs the duration of contraction in mammalian hearts. The major features of the altered contraction include: prolonged time to peak tension and time to half relaxation in the isometric contraction, and prolonged time to peak shortening and diminished shortening velocity in the isotonic contraction (5-9). The above changes may reflect, in part, changes in excitation-contraction coupling which may be caused by alteration of contractile proteins, changes in the intracellular action potential, and/or impaired energy delivery.

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Premature failure in ventricular performance in acute (<3 days) diabetes has been shown to be associated with inability of the heart to utilize physiologic concentrations of glucose as substrate for energy production (10). Higher concentrations of glucose or added insulin cause improvement. The ventricular contractile failure in chronic diabetes is not reversible by glucose or acute insulin treatment as is the case in acute diabetes. The impaired cardiac performance in chronic diabetes (>3 weeks) is reversible, however, with chronic insulin treatment(11). Changes in myofibrillar myosin structure resulting in lower ATPase activity have been suggested as causative in chronic diabetic cardiomyopathy(12-17). Prolonged contraction time, decreased rate of contraction.and relaxation seen in diabetic cardiomyopathy, all show a pattern of changes that could be observed when the energy delivery via phosphocreatine to the site of contraction is disrupted (18-20) and not just by decrease in myofibrillar ATPase activity which apparently could not affect relaxation as it affects contraction. There has been no investigation to date of the role of the phosphocreatine energy shuttle (21) in diabetic heart, although the importance of this energy transfer system in the process of excitation-contraction is recognized (22). It has also been shown that protein synthesis and muscle growth depend upon the phosphocreatine energy shuttle (22,23). Hence, any lesions or deterioration of the shuttle components could affect both growth and function of muscle. We therefore decided to study mitochondrial CPK in the heart of streptozotocin diabetic rat.

MATERIALS AND METHODS

180-200gm Male Sprague-Dawley rats were divided into two groups, control and streptozotocin injected. 60 mg streptozotocin (in 4.5 mM citrate buffer, pH =7.4)/kg body weight was injected into the tail vein after the rats had been fasted for 24 hours. Control and diabetic rats (10-11 days after

injection) were anesthetized with phenobarbital. The hearts were removed quickly and transferred into homogenizing medium containing, 0.35M mannitol, 0.1mM EDTA, 10mM Tris-HCl buffer, pH=7.2, at 4 degree C.

Mitochondria were isolated from ventricular tissue as described before (24) and suspended at about 2.5 mg mitochondrial protein/ml in a medium containing the same components as the homogenizing medium with 1% dialyzed BSA.

Atria and small pieces of ventricle were homogenized in 100mM Tris-HCl buffer, pH= 7.4, for determination of total CPK and AK activities in the direction of ATP production as has previously described (25-27). Protein was determined by the modified Lowry's method (28).

Mitochondrial oxygen uptake was determined (24) with either 300 uM ADP or 100 uM ATP in the presence or absence of 25 mM creatine.

RESULTS

10-11 day diabetic rats had 45±5% decrease in total body weight and their hearts seemed smaller to the same extent. Although the total weight of the heart was not determined, the total protein of the atria was measured and showed a drop of 48±11% in diabetic rat heart. Ventricles from diabetic heart yielded 46±6% less mitochondria than from control.

Although the total CPK activity of the homogenate made from ventricular or atrial tissue showed little difference, the mitochondrial CPK activity was 32±7% less in diabetic than in control heart. There were no differences between the adenylate kinase (AK) activity of control and diabetic heart determined either in the total homogenate or in mitochondria (Table 1).

Respiration of the diabetic heart mitochondria in the presence of ADP was reduced to 59±10% of that of control (table 2). The difference between the respiration rate of diabetic and control mitochondria in the presence of ATP was smaller (23±9% drop) due to the lower respiratory control obtained with diabetic mitochondria (3 for control verses 1.8 for diabetic). Addition of 25 mM creatine to the medium did not stimulate oxygen consumption, in the presence of ADP, by either control or diabetic mitochondria. Creatine (25 mM) stimulated

Table 1. CPK and AK activity of total homogenates and mitochondria

	CPK		AK	
	Total	Mito.	Total	Mito.
Control	1.60± 0.31	2.56± 0.13	1.32± 0.26	0.15± 0.03
Diabetic	1.37± 0.26	1.75± 0.18	1.31± 0.27	0.14± 0.04
% Reduction	14 NS	32± 7	0	0

The activity is shown as uM ATP/min/mg protein. Mito.= mitochondria. NS = not significant. Data are shown as mean ± SD. n=8 and P<0.001

the control mitochondrial oxygen consumption in the presence of ATP 2.6 times in comparison to 1.4 times in diabetic mitochondria (table 2). In some diabetic mitochondrial preparations in which

Table 2. Comparison of respiration rates of control and diabetic rat heart mitochondria

	ngm Atoms Oxygen / Min / mg Protein		
	Control	Diabetic	% Drop
None	73± 2	75± 7	0
ADP	391± 55	158± 17	* 59± 10
ADP+Cr	358± 74	160± 17	* 54± 14
Cr	76± 13	68± 11	11± 1
ATP	116± 6	90± 16	23± 9
ATP+Cr	306± 4 (2.6)	125± 1 (1.4)	* 59± 1
ATP **	185± 12	155± 9	16± 4
ATP+Cr **	307± 5 (1.7)	155± 6 (1)	

Mitochondrial oxygen uptake was determined with either 300uM ADP or 100uM ATP in the presence or absence of 25 mM creatine. * show that the data are highly significant ($p < 0.001$). ** are the data from mitochondrial preparations with low respiratory control (2.1 for control, 1.03 for diabetic, n=2). The number in parenthesis shows the folds increase in respiration rate with ATP by creatine. n=4. Data are shown as Mean ± SD.

the respiratory control was less than 1.2, addition of creatine did not stimulate the diabetic mitochondrial respiration and addition of ADP also was not able to stimulate it further (Table 2). In this case the respiratory rate of the diabetic mitochondria was already maximal (155 ngm atoms oxygen/min/mg protein) at less than the half of the control value. The control mitochondrial preparation never showed a respiratory control of lower than 2 and creatine was always able to stimulate respiration in the presence of ATP.

CONCLUSION

The present work centers around the role of mitochondrial CPK in regulation of energy production and its deficiency in the diabetic heart. In the present work chronic diabetes was associated with growth retardation, lower mitochondrial oxidative capacity, lower mitochondrial CPK activity and less ability of creatine to stimulate mitochondrial respiration.

The primary role of the mitochondria is to supply the cardiac cells with ATP which is delivered as phosphocreatine through the phosphocreatine energy shuttle for contraction and for the maintenance of ion homeostasis, protein synthesis, as well as for other endergonic cellular functions (23,29). This is accomplished through close interaction among mitochondrial CPK, oxidative phosphorylation and adenine nucleotide translocase. The present work shows that chronic diabetes diminishes mitochondrial oxidative phosphorylation capacity, and causes a drop in mitochondrial CPK activity, most strikingly in the loss of the ability of creatine to stimulate oxidative phosphorylation.

The mitochondrion has been shown to be the central terminus of the phosphocreatine energy shuttle (23,29). In the diabetic heart the drop in mitochondrial CPK activity and its

insensitivity to creatine could be responsible for a drop in cardiac energy delivery. Both the loss of respiratory control by ADP and the failure of energy delivery through the shuttle might be caused by partial failure of the mitochondrial Krebs Cycle resulting from a deficiency of insulin. This is a likely possibility in view of the recent demonstration of the mitochondrial Krebs Cycle as the ultimate site of insulin action (30). Thus, the final step of energy production by mitochondria, i.e. generation of phosphocreatine at the energy production site of the phosphocreatine energy shuttle (21), seems to be lower in diabetic heart than in control. This could be an important factor in cardiomyopathy and growth retardation seen in the diabetic mammalian heart. If there is a defect in localized CPK activity at the energy utilizing sites of the phosphocreatine energy shuttle, it could further contribute to the cause of diabetic cardiomyopathy. We are presently investigating this possibility.

We did not detect any significant changes in AK activity of either total homogenates or mitochondrial preparations from diabetic or control hearts. This makes creatine phosphokinase the focus for further investigation of diabetic cardiomyopathy since AK, which we have shown to be essential for optimal muscle function (31), is not impaired in the diabetic heart.

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