Manifestation of Adaptive Changes during Combined Development of Postinfarction Remodeling of the Heart and Diabetes Mellitus

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 8, pp. 132-135, August, 2010
Original article submitted October 27, 2009

Oxidative phosphorylation in isolated cardiomyocytes was studied under conditions of postinfarction remodeling and diabetes mellitus. Oxidation–phosphorylation uncoupling in the mitochondria in this disease combination was less pronounced than in each of these diseases alone. Combined development of the diseases was paralleled by less severe hyperglycemia and myocardial hypertrophy and lesser body weight loss. Presumably, combination of coronary occlusion and diabetes mellitus stimulates the adaptive changes in cardiomyocytes as early as at the level of mitochondrial energy metabolism.

Key Words: *mitochondria; postinfarction remodeling; diabetes mellitus*

Metabolic changes in cardiomyocytes in chronic diseases of the heart largely determine the prognosis for these diseases. Heart remodeling after coronary catastrophe is paralleled by disorders in ATP synthesis in cardiomyocyte mitochondria [14]. The disorders in mitochondrial function are triggered directly under the effect of hypoxia and indirectly through stress reaction, mediating the development of energy disorders [4,5]. Such metabolic disorders as accumulation of fatty acid metabolites, blockade of oxidative phosphorylation and glycolysis, accumulation of triglycerides in the myocardium, are characteristic of not only ischemic, but also of diabetic involvement of the myocardium [9]. Inhibition of glucose oxidation and transfer of cell energy metabolism to utilization of free fatty acids as the main substratum increase oxygen deficit in the myocardial, which inevitably impairs the pumping function of the heart.

Combination of coronary disease and diabetes mellitus has become a rather frequent condition in recent years requiring a special approach to the treatment of these patients [9,12]. However, specific features and regularities of metabolic changes developing in combined disease remain largely unclear.

We evaluated the effects of postinfarction remodeling and diabetes mellitus on oxidative phosphorylation in isolated cardiac mitochondria.

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MATERIALS AND METHODS

The study was carried out on 32 adult male Wistar rats. Four groups of 8 animals each were formed. Group 1 were intact animals, group 2 were animals with induced diabetes mellitus, group 3 were animals after coronary occlusion, and group 4 were rats in which diabetes mellitus was induced 2 weeks after coronary occlusion. Animals of groups 2 and 4 were studied 4 weeks after diabetes induction.

Diabetes mellitus was induced by a single intraperitoneal injection of 60 mg/kg streptozotocin (Sigma) diluted *ex tempera* 0.01 mol/liter in citrate buffer (pH 4.5) [1]. Diabetes mellitus was verified by a 4.5 times increase of blood glucose concentration and 56% body weight loss ($p<0.05$) in comparison with animals injected with citrate buffer. Serum glucose concentration was measured by enzyme colorimetric test (Biocon Diagnostic).

0007-4888/10/1502-0172

Coronary occlusion was modeled by ligation of the upper third of the left descending coronary artery as described previously [3].

Heart mitochondria were obtained by standard differential centrifugation in a medium containing 300 mM sucrose, 10 mM EDTA, and 8 mM Tris (pH 7.4) [14]. The mitochondria were suspended in 250 mM sucrose solution.

The rate of oxygen consumption by mitochondria was evaluated by polarography using a Clark electrode. The measurements were carried out in a medium (pH 7.4) containing (in mM): 300 sucrose, 10 KCl, 5 KH_2PO_4, 5 succinate, 1 EGTA, 1.2 $MgCl_2$, and 5 Tris; 100 μM ADP or 15 μM bromophene acylbromide (BPB) were added. All reagents were purchased from Sigma and ICN.

Respiratory control (RC) was determined as the ratio of respiration rate at maximum ATP synthesis and respiration rate in the absence of ATP synthesis [8].

Oxygen consumption rate is presented in nM O_2/min/mg protein. Protein concentration in a sample was evaluated by the standard Lowry's method.

All the data are presented as mean±standard error of the mean, the significance of the results was evaluated by nonparametric Mann–Whitney *U* test.

RESULTS

The results reflecting the initial mitochondrial respiration rate in the studied groups are presented in Table 1. This parameter in all experimental groups significantly surpassed the intact control. It increased more than 4-fold in diabetes (group 2) and more than 3-fold after coronary occlusion (group 3), but only 2-fold after combined disease. Increased oxygen consumption in these groups can be due to the protonophore effects of fatty acids [7] and hence, uncoupling of oxidative phosphorylation. Addition of ADP markedly decreased RC in groups 2 and 3. This confirms uncoupling of oxidation and phosphorylation processes in these diseases. A different result was observed in similar experiments on mitochondria isolated from cardiomyocytes of animals with combined disease. The initial oxygen consumption in this group was just 2-fold higher than intact animals, while after ADP addition RC approximated the value observed in intact animals.

It is known that many cardiovascular diseases are associated with high activity of endogenous phospholipases and hence, accumulation of free fatty acids [6]; this, in turn, provokes uncoupling of oxidation and phosphorylation in mitochondria [7]. This isé-n line with our data indicating that inhibition of phospholipase A_2 with BPB reduces the rate of oxygen consumption by rat cardiomyocytes after coronary occlusion to a level observed in normal cells [2].

In our study we also observed marked inhibition of oxygen consumption by cardiomyocyte mitochondria in the presence of BPB [10] in group 3 animals, while in group 2 this parameter remained high (Table 1). A similar study on mitochondria from animals with combined disease (group 4) has again shown a pronounced reaction to BPB. Importantly that inhibition of oxygen consumption in this group was even more pronounced than in group 3.

Hence, these data suggest that energy metabolism in cardiomyocytes is less disordered in combined disease.

According to modern concepts, hypoxia accompanies any disease and is a result of oxygen deficiency and/or effects of toxins [4,5], which leads to disorders in the mitochondrial functions and is the case in both diseases in our study. Disorders in the aerobic synthesis of energy and inhibition of energy-dependent metabolic functions of cell membranes are paralleled by changes in the glycolytic pathway of ATP formation. Its initial activation and lactate accumulation of is a process alternative to oxidative phosphorylation. However, persisting oxygen deficiency leads to glycolysis inhibition [4].

Presumably, this effect is due to the cascade of metabolic changes in mitochondria. Initially (at the bioenergetic hypoxia stage), changes in the mito-

TABLE 1. Oxygen Consumption Rate and RC in Rat Heart Mitochondria in Different Experimental Models (*M*±*m*)

Group	Oxygen consumption rate, nM O_2/min/mg protein		
	initially	in the presence of BPB	RC
1	10.5±1.8	10.6±1.4	3.4
2	44.7±3.8	38.0±2.7	2.0 (70%)
3	35.2±3.5	21.1±2.4	1.9 (78%)
4	20.9±1.5	11.6±1.5	2.3 (48%)

Note. Percentage of RC decrease compared to group 1 (100%) is shown in parentheses.

TABLE 2. Disease Effects on Changes in Glucose Level, Body Weight, and Heart Weight in Laboratory Animals

Group	% of changes in parameters *vs.* group 1		
	glucose level	body weight	heart weight
2	285.7	86.0	17.0
3	16.7	23.1	95.3
4	157.14	34.8	5.0

Note. All values are presented in percent of the parameters in intact animals (group 1) taken for 100%.

chondrial respiratory chain are paralleled by a drop of NADH-dependent oxidation activity, this leading to stimulation of compensatory alternative metabolic pathways [4,5]. The most rapid alternative pathway is the succinate oxidase oxidation, which is stimulated only through elevation of SDH activity in the mitochondria.

Since these events are not tissue-specific and, moreover, are components of nonspecific stress reaction [4], a combination of diseases can be associated with the summary effect of this compensation further reducing manifestations of pathophysiological phenomena.

Our data are in line with the results of other authors who demonstrated an increase of myocardial resistance to ischemia in animals with the initial stages of streptosotocin-induced diabetes [11].

Comparison of body weights, heart weights, and blood glucose levels in animal groups showed possible functional significance of the detected differences in energy metabolism (Table 2). Animals with combined disease (group 4) developed less pronounced changes in glucose level and body and heart weights than animals with diabetes mellitus (group 2). We should like to emphasize a lesser manifestation of heart hypertrophy (in addition to the increase of blood glucose level) in combined disease (group 4) in comparison with animals subjected to coronary occlusion (group 3).

However, it is worthy of note that more favorable status of energy metabolism detected in animals with combined disease cannot last long and is fraught with failure of adaptation. The prognosis for patients with this disease combination is far from optimistic. Clinical observations indicate that combined disease is really life threatening [12]. However, our results suggest that the adaptive changes in cardiomyocytes in chronic diseases are realized to a certain measure at the mitochondrial energy metabolism level, in humans as well.

Hence, these data indicate that combined effects of the ischemic factor and diabetes mellitus on the rat myocardium are initially characterized by a more benign time course of pathophysiological manifestations, due to (most likely) nonspecific stimulation of alternative pathways of oxidation and rapid resynthesis of ATP in mitochondria.

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