Role of Phospholipase A_2 in Activation of Isolated Cardiomyocyte Respiration in Postinfarction Cardiosclerosis

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The rate of oxygen consumption by isolated cardiomyocytes was studied in rats with experimental postinfarction cardiosclerosis. The increase in oxygen consumption under these condition was comparable to that in melittin- and arachidonic acid-induced activation of phospholipase A_2 in cardiomyocytes of intact animals. Bromophenacyl bromide inhibition of phospholipase A_2 in cardiomyocytes of rats with postinfarction cardiosclerosis led to reduction of oxygen consumption rate to values characteristic of intact animal cardiomyocytes. The results confirm the hypothesis according to which high oxygen consumption in postinfarction cardiosclerosis is related to increased activity of phospholipase A_2.

Key Words: *cardiomyocytes; postinfarction cardiosclerosis; phospholipase A_2; melittin; bromophenacyl bromide*

Homeostasis disorders and structural changes in cardiomyocyte membranes are observed in different pathologies, including various stages of heart failure forming after myocardial infarction. The content of free fatty acids increases in various ischemic syndromes [5], and activation of lipolysis processes under the effect of endogenous factors can promote this increase. Deacylation/reacylation reactions involved in fatty acid metabolism are among the processes maintaining the membrane homeostasis. It is assumed that shifts in the membrane remodeling process leading to deacylation/reacylation imbalance are the key factor in fatty acid release, presumably due to increased activity of membrane phospholipases [6]. It is known that reduced content of ATP in hypoxia promotes activation of endogenous phospholipases, degradation and loss of membrane phospholipids, increased membrane fluidity and its selective permeability [4].

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Pathological accumulation of lisophospholipids can be associated with activation of phospholipase A_2 (PLA). This phospholipase generates lysolecithins and fatty acids, including arachidonic acid. Arachidonic acid transformation leads to the formation of a series of intracellular lipid agents, which, in turn, regulate activities of membrane-bound enzymes, including PLA.

Here we studied the involvement of PLA and arachidonic acid into regulation of oxygen consumption by cardiomyocytes in postinfarction cardiosclerosis (PICS) in experimental rats.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (250-300 g). In order to induce PICS, the animals narcotized with ether, the chest was opened, and pericardiotomy and ligation of the left anterior descending coronary artery were carried out. The wound was sutured layer-by-layer after air removal from the thorax. Further studies were carried out after 40 days, when the animals developed PICS. Morpho-

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logical changes in the myocardium were evaluated by histological examination [3].

Cardiomyocytes were obtained after continuous perfusion of the heart with a combination of proteolytic enzymes; we used our modification of the method for enzymatic isolation of cardiomyocytes [2]. Cardiomyocytes isolated from the hearts of intact animals served as the control.

The rate of oxygen consumption was evaluated during the 1st hour after derivation of cardiomyocyte suspension by polarography using Clark's electrode. The cells were incubated in Krebs—Henseleit buffer with 2 mM $CaCl_2$ (pH 7.4). The measurements were carried out in a vessel incubated in a thermostat at 27°C with constant stirring on a magnetic mixer. Protein concentration was measured by the method of Lowry. Oxygen consumption rate is presented in nmol O_2/min/mg protein. All the data are presented as means±standard error of the mean. Ten animals were used at each stage of experiment. The significance of differences was evaluated using Student's *t* test.

RESULTS

The initial rate of oxygen consumption by isolated cardiomyocytes in PICS more than 4-fold surpassed that in intact rat cardiomyocytes (Fig. 1). This result is in line with modern notions on the metabolic aftereffects of myocardial ischemia, leading to accumulation of fatty acid metabolites, blocking of glucose oxidative phosphorylation and glycolysis, and to increased content of triglycerides in the myocardium. The excess of free fatty acid and glucose oxidation inhibition are responsible for high oxygen consumption by the myocardium, which is paralleled by augmenting energy deficit [8]. Importantly that this reaction is generalized in PICS, and an appreciable reduction of energy resources was shown even for intact sites of the myocardium [7]. Energy deficit in cells can lead to activation of endogenous phospholipases capable of cleaving fatty acids from cell membrane structures, primarily arachidonic acid. The rate of oxygen consumption in cardiomyocytes isolated from the hearts of rats with PICS was comparable to that in intact rat cardiomyocytes during stimulation of their respiration by arachidonic acid (Fig. 1). However, addition of arachidonic acid to the incubation medium did not increase cardiomyocyte respiration rate in PICS (Fig. 1). This result can be explained by the fact that the increase in oxygen consumption rate in PICS seems to be realized through a mechanism linked with arachidonic acid. It was shown that accumulation of fatty acid was slow at the initial stage of

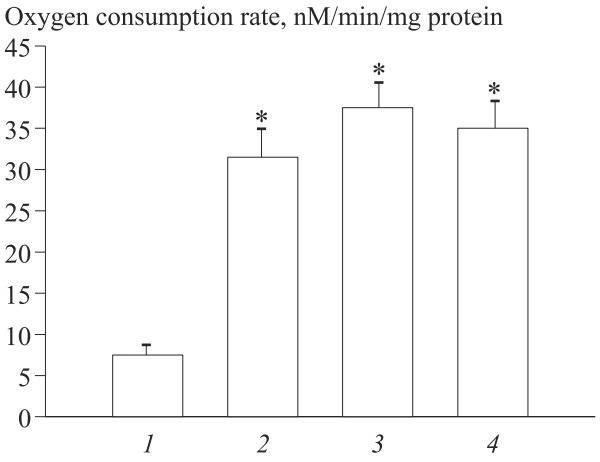

Fig. 1. Effect of arachidonic acid (AA) on oxygen consumption by cardiomyocytes from intact rats and rats with PICS. *1*) cardiomyocytes of intact rats; *2*) PICS; *3*) cardiomyocytes of intact rats+AA (45 μM); *4*) PICS+AA (45 μM). Here and in Fig. 2: **p*<0.05 compared to *1*.

ischemia and fatty acids are cleaved only from membrane phospholipids [9]. At later stages of ischemia, lysophospholipid concentrations increased [10] and accumulation of toxic products of fatty acid degradation created conditions for higher oxygen consumption by the myocardium, this increase being unrelated to the increase in its mechanical work.

We previously showed that addition of arachidonic acid stimulated PLA in isolated intact rat cardiomyocytes [1]. Presumably, under conditions of PICS energy deficit caused by insufficient oxygen supply leads to an increase in PLA activity. Activation of PLA results in arachidonic acid release, which, in turn, also stimulated PLA thus closing the "vicious circle", which shifts the balance in membrane remodeling processes towards accumulation

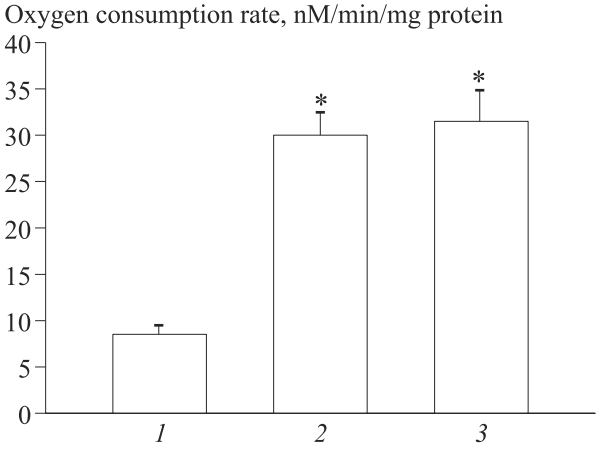

Fig. 2. Comparison of melittin-stimulated respiration in cardiomyocytes from intact rat and cardiomyocytes from rats with PICS. *1*) intact rat cardiomyocytes; *2*) intact rat cardiomyocytes+melittin (0.5 μg/ml); *3*) PICS.

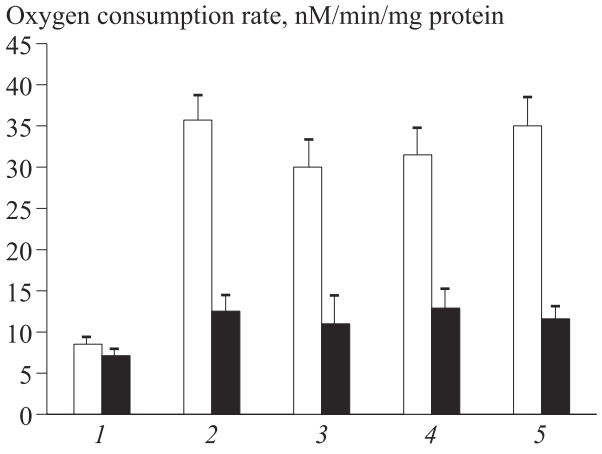

Fig. 3. Comparison of BPB effects on initial and stimulated respiration of cardiomyocytes. Light bars: control; dark bars: BPB. *1*) intact rat cardiomyocytes; *2*) 45 μM AA; *3*) 0.5 μg/ml melittin; *4*) PICS; *5*) PICS+45 μM AA.

of lysophospholipids. In order to verify this hypothesis about PLA involvement into the processes leading to an increase of oxygen consumption by isolated cardiomyocytes in PICS, we carried out experiments with PLA activator melittin and PLA inhibitor bromophenacyl bromide (BPB).

Addition of melittin (0.5 μg/ml) to the incubation medium resulted in a more than 4-fold increase in the rate of oxygen consumption by cardiomyocytes in intact animals, the level of this parameter reaching the value in cardiomyocytes of patients with PICS. Arachidonic acid (45 μM) and melittin produced similar stimulatory effects on respiration of intact animal cardiomyocytes (Fig. 1, 2). Results of experiments with BPB confirmed our hypothesis. Respiration of intact cardiomyocytes stimulated with arachidonic acid or melittin returned virtually to the initial level after addition of BPB (15 μM; Fig. 3). It is remarkable that BPB *per se* virtually did not modify the rate of oxygen consumption by intact cardiomyocytes. However, addition of BPB to cardiomyocytes of animals with PICS reduced initially high rate of oxygen consumption to the level of intact cells (Fig. 3). It is noteworthy that the decrease in the rate of oxygen consumption was virtually the same in all cases of BPB use.

Hence, cardiomyocytes in experimental PICS are characterized by increased oxygen consumption unrelated to their inotropic activity. These results confirm the modern notions according to which the increase of cardiomyocyte oxygen consumption in PICS is caused by PLA activation. However, the route and the order of events leading to PLA activation in PICS remain unknown. First, it can result from energy deficit in myocardial cells because of oxygen deficiency induced by coronary artery ligation and degeneration of the myocardial tissue. Second, the processes impairing mitochondrial function and realized during the direct effect of hypoxia on the cell or mediated through nonspecific stress reaction eventually leading to energy disorders can serve as the factors initiating PICS development [4].

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