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EFFECT OF ISCHEMIA AND POSTISCHEMIC REPERFUSION ON FATTY ACID OXIDATION IN CARDIAC MITOCHONDRIA

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Postischemic reperfusion (PIR) of the myocardium has been shown [3, 10] to help to restore the viability of its cells and its contractile function. During PIR further ultrastructural damage to the myocardial cells and a decrease in the concentration of ATP and total adenine nucleotides, as well as a sharp increase in release of cytoplasmic enzymes [12], are also observed. The effect of PIR on mitochondrial function has received very little study. Kane et al. [6] found that PIR for 2 h after occlusion of the coronary artery for 15-180 min does not restore respiration of cardiac mitochondria (Mc) with glutamate and malate. Moreover, even more marked depression of respiration than during continuous occlusion was observed after ischemia for 3 h followed by PIR. According to our data, occlusion of the coronary artery for 1 h followed by PIR for 24 h led to much lower activity of fatty acid oxidation than in the control [2]. However, it was not clear whether reperfusion promotes normalization of functions of Mc or aggravates their injury.

To study this problem the effect of continuous ischemia and PIR on activity of individual stages of the fatty acid oxidation enzyme system was compared.

EXPERIMENTAL METHOD

Rabbits weighing 2.5-3 kg were used. Myocardial ischemia was induced by continuous and temporary (1 h) occlusion of the anterior descending branch of the left coronary artery [13]. Material for isolation of Mc was taken after 1 and 4 h in the case of continuous occlusion, and 3 h after occlusion for 1 h followed by PIR. The method of isolation of Mc was described previously [2]. Respiration of Mc was recorded polarographically at 37°C in incubation medium containing 0.15 M KCl and 0.005 M KH_2PO_4, pH 7.4. Activity of the β-oxidation system was studied in the same medium by the method described by Bremer and Davis [4]. Protein was determined by a modified biuret method [1]. Reagents: palmitoyl CoA (P-CoA) was from Sigma, USA, L-carnitine and L-palmitoyl carnitine (PCar) (from Japan) were generously provided by Dr. J. W. de Jong and the firm "Otpuska," to whom the authors are grateful.

EXPERIMENTAL RESULTS

Depending on the length of their carbon chain, fatty acids are oxidized by enzyme systems of different complexity. Comparative analysis of the velocities of oxidation of P-CoA, PCar, and acetate gives information about the state of oxidative enzymes specific for short and long carbon chains.

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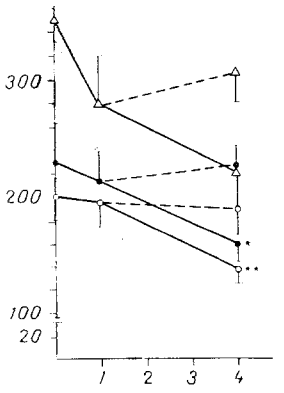

Fig. 1

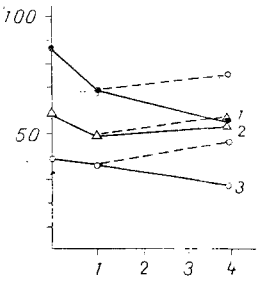

Fig. 2

Fig. 1. Effect of continuous occlusion of coronary artery and PIR on velocity of oxidation of 2 mM acetate (triangles), 21 μM PCar (filled circles), and 21 μM P-CoA (empty circles) in rabbit heart mitochondria. Here and in Fig. 2, continuous line denotes continuous occlusion, broken by PIR. Oxidation of acetate was activated by addition of 400 μM ADP. During oxidation of P-CoA and PCar, medium contained 2 mM L-carnitine, and oxidation was activated by addition of 4 mM ADP. *P < 0.01, **P < 0.001. Abscissa, duration of occlusion (in h); ordinate, velocity of mitochondrial respiration in state 3 (in nanoatoms O_2/min/mg protein).

Fig. 2. Effect of continuous occlusion of coronary artery and PIR on rate of acetate utilization in tricarboxylic acid cycle (1), rate of its utilization during β-oxidation (2), and complete oxidation of PCar (3). Methods of calculating velocities of utilization and consumption of acetate were described previously [2, 4]. Abscissa, duration of occlusion (in h); ordinate, rate of utilization or formation of acetate (in nmoles/min/mg protein).

It will be clear from Fig. 1 that with an increase in the duration of coronary artery occlusion the velocity of respiration of Mc in state 3 with all substrates used fell progressively, and by a very similar degree. After 4 h of occlusion these changes were statistically significant. The velocity of respiration with PCar, both in the control and during ischemia, was higher than its velocity with P-CoA, and activity of acetate oxidation was the highest. The velocity of respiration of Mc with acetate characterizes activity of the tricarboxylic acid cycle (TAC). The rate of utilization of acetate, in nanomoles/min/mg protein during oxidation of acetate and the rate of its formation during complete oxidation of PCar were calculated from the results in Fig. 1. The results of these calculations are given in Fig. 2. It follows from them that the rate of utilization of acetate in the TAC, both in the control and during ischemia, was much higher than the rate of its formation. The results indicate that external carnitine-palmitoyltransferase limits the velocity of oxidation of P-CoA, but TAC and the respiratory chain do not limit oxidation of p-CoA and PCar.

PIR helps to promote some recovery of respiration of Mc with acetate and PCar, but in this case activity of P-CoA oxidation is unchanged. The beneficial effect of PIR on respiration of Mc becomes evident on comparison with the effect of continuous occlusion for 4 h (Fig. 1). Inhibition of mitochondrial function depends on the duration of PIR [5] and on the degree of previous reduction of the coronary circulation [14]. With longer PIR (24 h, after 1 h of occlusion) considerable depression of respiration of Mc with these same substrates is observed [2]. Consequently, the positive effect of PIR for 3 h on mitochondrial function is evidently temporary.

Pande [8] concludes that the β-oxidation system in cardiac Mc limits oxidation of palmitate, but Bremer and Davis [4] came to the opposite conclusion. We found that the velocity of acetate formation from PCar in the β-oxidation system (Fig. 2, 2) always exceeds that during complete oxidation of PCar (Fig. 2, 3). Consequently, the results of the present experiments agree with the findings of Bremer and Davis [4]. The results in Fig. 2 also show that

during ischemia and PIR respiration of Mc during β-oxidation of PCar remains substantially ununchanged. Judging from activity of mitochondrial respiration during β-oxidation of PCar, electron-transport activity of the respiratory chain and the oxidative phosphorylation system do not limit β-oxidation. In the experiments described above optimal substrate concentrations were used. An interesting feature of Mc when damaged by ischemia came to light when low concentrations of PCar (2 μM) were used. In that case the added PCar was oxidized until all the oxygen in the cuvette was used up, so that the ΔO/Δ palmitoyl ratio, characterizing completeness of oxidation of the palmitoyl residue, the theoretical value of which is 46, could be calculated. Clearly, during continuous occlusion of the coronary artery oxidation of PCar was inhibited and the ratio ΔO/Δ palmitoyl fell. During PIR these parameters were restored to some degree. By contrast with high concentrations of PCar (about 30 μM) the respiration rate in the presence of low PCar concentrations is limited by the acyl-transfer system [9]. One possible cause of the reduction in the velocity of oxidation of 2 μM PCar is disturbance of its transport through the mitochondrial membrane. According to Levitsky and Skulachev [7] PCar transport activity depends on the electrochemical hydrogen ion gradient. It seems probable that its decline during myocardial ischemia [13] may have some effect on PCar transport and, consequently, on the rate of oxidation of this substrate.

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