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The role of long-chain acyl-CoA in the damage of oxidative phosphorylation in heart mitochondria

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The aim of this investigation was to study the effect of intramitochondrial acyl-CoA on the respiration of rabbit heart mitochondria over the whole range of stationary respiratory rates between States 4 and 3. The creatine phosphokinase system was used for stabilization of extramitochondrial adenine nucleotide concentration. It was shown that acyl-CoA depressed respiration more effectively in the intermediate range of respiration between States 4 and 3. The effect of acyl-CoA was negligible near State 4 and in State 3. These data are in line with our previous results concerning the dependence of the adenine nucleotide translocator control coefficient on the rate of mitochondrial respiration. Thus, our data suggest that long-chain acyl-CoA may regulate oxidative phosphorylation in heart mitochondria in vivo.

Adenine nucleotide translocator; Long-chain acyl-CoA; Oxidative phosphorylation; (Heart mitochondria)

1. INTRODUCTION

Activity of oxidative phosphorylation significantly decreases under conditions of myocardial ischemia concomitantly with accumulation of long-chain acyl-CoA. It is well known that these esters are potent inhibitors of ANT [1]. It has been proposed that inhibition of adenine nucleotide transport by acyl-CoA is one of the steps in role in disturbance of the energy metabolism of the ischemic myocardial cell [2]. At the same time, data exist which at first glance seem to contradict the above-mentioned hypothesis. It was shown that a 3-fold increase in intramitochondrial acyl-CoA concentration only slightly depressed mitochondrial respiration and oxidative phosphorylation in State 3 [3]. Some investigators deny the possibility of regulation of oxidative phosphorylation in myocardium by long-chain acyl-CoA in vivo [3,4].

We have previously shown that the control coefficient of ANT on the rate of heart mitochondria respiration (succinate as substrate) in State 3 is equal to zero [5]. This may be the reason why the effect of long-chain acyl-CoA on mitochondrial respiration is negligible in this metabolic state. General conclusions about the effect of acyl-CoA on mitochondrial respiration in vivo are based on investigations in State 3. Such generalization seems to be unfounded, because mitochondria in the cell are in the intermediate states between States 4 and 3.

The aim of this investigation was to study the effect of elevated intramitochondrial acyl-CoA concentration on mitochondrial respiration throughout the range of stationary rates between States 4 and 3 under conditions of stabilized extramitochondrial phosphorylation potential.

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Abbreviation: ANT, adenine nucleotide translocator

2. MATERIALS AND METHODS

Rabbit heart mitochondria were isolated with trypsin as described [6]. The concentration of long-chain acyl-CoA in

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mitochondria was elevated over the 1–1.5 h incubation (0°C) with palmitoyl-L-carnitine (10 nmol/mg protein) and rotenone (2 μM) in a medium containing 180 mM KCl and 20 mM Tris-HCl (pH 7.35). The concentration of mitochondrial protein was 40 mg/ml. Mitochondria incubated in the same medium with rotenone only were used as controls. Mitochondrial respiration was measured polarographically. Different stationary rates of mitochondrial respiration were adjusted by varying creatine phosphate in the medium [5]. Mitochondrial protein concentration was determined by the biuret method. Long-chain acyl-CoA content was estimated spectrophotometrically [7].

3. RESULTS AND DISCUSSION

For producing steady-state conditions, the incubation medium for mitochondria is supplemented with an ADP-regenerating system, usually hexokinase and glucose or F_1-ATPase [8,9]. Different stationary rates of mitochondrial respiration between States 4 and 3 are adjusted by adding various amounts of the ADP-regenerating system. Because of the inevitable limit of hexokinase or F_1-ATPase at intermediate rates of respiration, flux control is exerted mainly by the ADP-regenerating system [9]. Under such conditions the resulting flux may not be influenced by the external effector, which affects only the mitochondrial processes, and has no effect on the ADP-regenerating system. Therefore, the usual ADP-regenerating system appears to be inadequate for investigation of effectors of oxidative phosphorylation under conditions which are close to physiological. The distinctive feature of our experiments was that the ADP-regenerating system did not control oxidative phosphorylation and at all states of mitochondria between States 4 and 3 the control of respiration was shared only among mitochondrial processes.

The concentration of intramitochondrial acyl-CoA after incubation of mitochondria with palmitoylcarnitine and rotenone increased from 0.19 ± 0.04 nmol/mg protein (mitochondria incubated with rotenone only) to 0.56 ± 0.02 (acyl-CoA-loaded mitochondria). There was no difference in the rate of uncoupled respiration between these mitochondrial preparations: 265 ± 16 and 278 ± 17 ngatom O/min per mg protein, respectively. Hence, the succinate-oxidase system of mitochondria was not affected by acyl-CoA.

Table 1 shows that the degree of inhibition of mitochondrial respiration by matrix acyl-CoA (expressed as percent of control) is dependent upon the stationary rate of mitochondrial respiration. As can be seen, the inhibitory effect of a given acyl-CoA concentration was about 6% near State 4, increased further to 17–20% for intermediate rates of mitochondrial respiration (60–90% of the rate in State 3) and decreased to 4% close to State 3.

Activation of mitochondrial respiration by L-carnitine may be the test for inhibition of ANT by acyl-CoA. Our experiments showed that the concentration of acyl-CoA decreased to 0.3 nmol/mg protein (n = 2) after 1 h incubation (0°C) of mitochondria with 4 mM L-carnitine. The rate of oxygen consumption by acyl-CoA-loaded mitochondria after such incubation increased close to the level of the control mitochondrial respiratory rate.

The dependence of the inhibitory effect of matrix acyl-CoA on the stationary rate of mitochondrial respiration appears similar to the corresponding dependence of the control coefficient of ANT on mitochondrial respiration [5]. However, there are noticeable differences. At a rate of respiration amounting to 30–40% of that in State 3, the control coefficient of ANT has a maximal value (about 0.7), which remains stable until a rate of about 80% of that of State 3, whereas the inhibitory effect of acyl-CoA increases in this region of mitochondrial respiration rates. This difference can be explained by differing sensitivities of ANT to acyl-CoA for various states of the oxidative phosphorylation system between States 4 and 3. Acyl-CoA from the cytosolic side competes with external adenine nucleotides for ANT, and from the matrix side, with internal adenine nucleotides. When mitochondrial ATP is exchanged with external ADP, intramitochondrial acyl-CoA competes with matrix ATP for ANT and depresses this mode of exchange. Obviously, acyl-CoA also inhibits the reverse exchange of mitochondrial ADP with external ATP, however ANT is far from thermodynamic equilibrium even at low respiratory rates, close to State 4, because of the electrogenic character of exchange, so it is possible to neglect the reverse exchange and influence of acyl-CoA on it. At higher stationary rates of mitochondrial respiration, the matrix ATP/ADP ratio and ATP concentration are lower, because the exchangeable pool of adenine nucleotides remains unchanged. The influence of

Table 1

Inhibitory effect of long-chain acyl-CoA on mitochondrial respiration

	Relative rate of respiration (%)						
	31–40	41–50	51–60	61–70	71–80	81–90	91–100
Inhibitory effect (%)	6.2 ± 4.8	11.0 ± 1.4	10.5 ± 0.7	17.5 ± 2.1	18.7 ± 2.8	19.5 ± 0.7	4.1 ± 3.4

Mitochondria (0.5 mg/ml) were incubated in a medium consisting of 10 mM Tris-HCl, 5 mM KH_2PO_4 (pH 7.2), 20 mM succinate, 1 μM rotenone, 10 mM dithiothreitol, 1 mM ATP, 4 IU/ml creatine phosphokinase, 0.5 mM free Mg^{2+} and various amounts of creatine, creatine phosphate and KCl (see [5] for details). Data from nine experiments are presented

intramitochondrial acyl-CoA on the direct exchange is kinetically equivalent to the increase in apparent K_m for matrix ATP. It has a greater effect on the exchange rate at low concentrations of ATP and correspondingly high rates of mitochondrial respiration than at high ATP and corresponding low respiratory rates. For this reason the sensitivity of ANT to intramitochondrial acyl-CoA increases monotonically, when the rate of respiration increases between States 4 and 3, following the corresponding increase in inhibitory effect of acyl-CoA on respiration.

It was shown [11] that accumulation of long-chain acyl-CoA in liver during ischemia and starvation inhibited the rate of respiration in State 3. This can be explained by the fact that the control coefficient of ANT in liver mitochondria oxidizing succinate is not equal to zero in this state, but has a value of about 0.3 [8,11].

Thus, the results obtained suggest that long-chain acyl-CoA accumulation in the matrix depresses the stationary rate of oxidative phosphorylation under conditions of stabilization of the extramitochondrial phosphorylation potential at physiological rates of heart mitochondrial respiration. The sensitivity of ANT to intramitochondrial acyl-CoA may increase in mitochondria of ischemic myocardium, as a consequence of the decreased content of exchangeable adenine nucleotides.

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