KINETIC DIFFERENCES BETWEEN MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION SYSTEMS OF THE INTACT AND ISCHEMIC HEART

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There is now considerable experimental evidence that the level of long-chain acyl-CoAs in the ischemic myocardium [6, 9, 14] and mitochondria (MC) of the heart [6, 8, 9] is increased, and that most of them are located in MC [6, 11]. It has also been shown that much of the acyl-CoA is oxidized on isolation of MC by the usual methods [6, 11]. This process is prevented by inhibitors of mitochondrial respiration [6, 11]. Acyl-CoA is known to inhibit adenine-nucleotide translocase (ANT) *in vitro* competitively to ADP [12]. This effect is observed both in cytoplasm [12] and on matrix [5, 7]. On the basis of measurements of the acyl-CoA concentration in the myocardium and respiration and ANT activity of isolated MC it has been suggested that inhibition of ANT by acyl-CoA is the key stage in disturbance of energy metabolism of the ischemic myocardial cell [4]. However, the results of that investigation can also be interpreted differently, especially if it is recalled that MC were isolated without respiration inhibitors.

The aim of this investigation was to study the effect of total myocardial ischemia on mitochondrial respiratory activity in the heart, the acyl-CoA concentration, and kinetic characteristics of the oxidative phosphorylation system. Since acyl-CoAs which accumulate in MC in ischemia are oxidized in the course of isolation, the MC were isolated by ordinary methods and also in medium containing rotenone.

EXPERIMENTAL METHOD

Myocardial ischemia was produced by autolysis for 30 min at 37°C [3]. The heart was rinsed briefly in ice-cold 0.9% KCl solution and divided into two parts: One part served as the control, the other was subjected to autolysis. MC were isolated as described in [2]. In some series of experiments, to prevent oxidation of acyl-CoA, a solution of rotenone (1 mg/g tissue) in ethanol was added to the homogenization medium. Activity of oxidative phosphorylation was determined pH-metrically [10] at 37°C in medium containing 0.3 M sucrose, 0.005 M KCl, and 0.005 M KH_2PO_4, pH 7.4. The concentrations of substances and other additives were: 10 mM succinate, 3 μM rotenone, 0.05-1 mM ADP, and 0.5 mg mitochondrial protein to 0.8 ml of medium. Respiration of MC was recorded polarographically at 37°C in the above-mentioned medium. The concentrations of additives were: 10 mM succinate, 3 μM rotenone, 100 μM ADP and 2,4-dinitrophenol (2,4-DNP), and 0.5 mg mitochondrial protein to 0.84 ml. Mitochondrial protein was determined by the biuret method and the concentrations of CoA-derivatives of long-chain fatty acids in MC isolated with rotenone were determined by the method in [15] (immediately after isolation of MC) on a model 557 spectrophotometer (Hitachi, Japan). Reagents: succinate was from Reanal, Hungary, 2,4-DNP from Serva, West Germany, and DL-carnitine, rotenone, and ADP were from Sigma, USA.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the rate of respiration of MC isolated without rotenone (experiment 1) in the presence of myocardial ischemia was reduced in state 3 by the same

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TABLE 1. Effect of Myocardial Ischemia on Mitochondrial Respiration in Rabbit Heart (M ± m)

Expt.	Experimental conditions	V_2	V_3	V_4	V_5	$V_3 - V_2$	V_3/V_2	$\frac{V_5}{V_3 - V_2}$
1	Control	261±41	615±63	322±57	733±76	354±44	2,47±0,5	2,08±0,11
	Ischemia	282±30	462±47*	296±7,0	520±26*	180±22*	1,70±0,07*	2,94±0,25*
		+8%	−25%	−11%	−29%	−49%	−31%	+41%
2	Control	223±17	385±28	256±12	503±49	164±20	1,80±0,04	2,83±0,10
	Ischemia	284±33	360±40	302±41	447±73	75±12,5*	1,25±0,03*	5,26±0,48*
		+27%	−6,5%	+18%	−11%	−54%	−30%	+86%

Legend. Rate of respiration expressed in nanoatoms oxygen/min/mg protein: V_2) rate of respiration before addition of ADP, V_3) the same after addition of ADP, V_4) the same after end of phosphorylation of ADP, V_5) rate of respiration uncoupled by 2,4-DNP. Changes expressed as percentages given relative to control. In experiment 1 MC were isolated without rotenone, in experiment 2 with rotenone. Number of tests: five in experiment 1, four in experiment 2, except V_5 and $V_5/(V_3 - V_2)$, in each of which there were three tests. Here and in Table 2 *P < 0.05.

TABLE 2. Effect of Myocardial Ischemia and Carnitine on Kinetic Constants of Oxidative Phosphorylation System and on Concentrations of Acyl-CoAs in Rabbit Heart MC (M ± m; n = 4-6)

Expt.	Experimental conditions	K_m, μM ADP	V_{max}, nmoles H^+/min/mg	Acyl-CoA, nmoles/mg
1	Control	31,5±3,5	639±54	
	Ischemia	32,8±3,2	411±20*	
			−36%	
2	Control	52±6,6	382±50	0,174±0,027
	Ischemia	125±13,3*	238±32*	0,260±0,028*
		+58%	−38%	+52%
3	Ischemia (control)	185±52	195±27	
	Ischemia + D,L-carnitine (25 mM)	96±30*	197±17	
		−49%		

Legend. Kinetic constants calculated by means of Lineweaver–Burk plots. Changes expressed as percentages given relative to corresponding control. In experiment 1 MC were isolated without rotenone, in experiment 2 with rotenone, in experiment 3 with rotenone + carnitine.

degree as the rate of uncoupled respiration. The most distinct decrease was observed in phosphorylating respiration ($V_3 - V_2$). The ratio between the rates of uncoupled respiration and phosphorylating respiration in this case was increased by 41%. A very similar picture of changes also was observed on isolation of MC with rotenone (experiment 2). In this case, however, the increase in the ratio of the rate of uncoupled respiration to phosphorylation was much greater, evidence of the relatively greater inhibition of phosphorylation than of uncoupled respiration. Some increase in the rates of respiration V_2 and V_4 was observed in this series of experiments.

The difference between the effects of ischemia on MC isolated with and without rotenone was revealed particularly clearly by a study of the kinetics of oxidative phosphorylation. It will be clear from Table 2 that the maximal rate of oxidative phosphorylation in MC damaged by ischemia was lowered equally irrespective of whether MC were isolated with rotenone (experiment 2) or without it (experiment 1). The value of the apparent Michaelis constant (K_m) for ADP of the oxidative phosphorylation system of ischemic MC isolated without rotenone was indistinguishable from the control (experiment 1) but much higher than the control when MC were isolated with rotenone (experiment 2). These results agree with the greater increase in the

ratio between the rate of uncoupled respiration and of phosphorylating respiration in experiment 2 (Table 1), evidence of accumulation of an inhibitor competitive for ADP in MC, which could in all probability be long-chain acyl-CoAs. The inhibitory effect of exogenous acyl-CoA is abolished by carnitine [12], and it is converted into acyl-carnitine, a compound which does not inhibit ANT. Stimulation of oxidative phosphorylation by carnitine is regarded as a specific test of inhibition of ANT by acyl-CoA [13]. Addition of L-carnitine (2 mM) to the incubation medium, however, had no significant effect on the rate of oxidative phosphorylation, measured pH-metrically in the presence of 100 μM ADP: This applies to both intact MC (control) and those damaged by ischemia. It is possible that during short-term incubation (under 1 min) too little carnitine is transported into MC.

In a separate series of experiments (experiment 3, Table 2) MC were isolated from ischemic heart tissue with rotenone and with rotenone and carnitine, and in this way the duration of contact between MC and carnitine was increased. In this case carnitine led to significant lowering of the value of K_m for ADP (Table 2). The results are evidence that the cause of competitive inhibition of oxidative phosphorylation in MC of the ischemic heart is inhibition of adenine-nucleotide transport by long-chain acyl-CoAs accumulating in MC during ischemia. Direct measurements of acyl-CoA in rabbit heart MC isolated with rotenone (separate series of experiments) showed an increase in their content during ischemia by 52% (Table 2). In this connection it is worth noting that palmitoyl-CoA does not inhibit ATPase of submitochondrial particles [1], and that its known inhibitory effect on certain other enzymes and carriers of oxidation substrates is much weaker than on ANT.

An increase in K_m for ADP of the oxidative phosphorylation system was observed in [1, 13] during uncoupling caused by aging of liver MC. In our experiments, however, uncoupling of oxidative phosphorylation (admittedly, to a less marked degree) in ischemic MC isolated without rotenone (experiment 1, Table 1) was unaccompanied by an increase in this parameter, in agreement with the results of investigations [6, 11] in which an increase in the acyl-CoA concentration was observed only when MC were isolated with rotenone.

A fall in the maximal velocity of oxidative phosphorylation during ischemia may be due to a decrease in oxidative and phosphorylating ability of MC and of the rate of adenine-nucleotide transport. The latter largely depends on the content of intramitochondrial adenine nucleotides and, in particular, of total ATP + ADP. In ischemia, this total is considerably reduced [11]. However, this has no effect on K_m for ADP transport [4].

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