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Effects of Apolipoproteins C on Oxidative Phosphorylation in Rat Liver Mitochondria

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It is shown that apoC-III, but not other apoC proteins, components of very low density lipoproteins (apoC-I, apoC-II, apoC-III), reduced the rate of mitochondrial respiration in various metabolic states. This effect depended on the dose of apoprotein, type of oxidized substrate, and the presence of Ca ions in the incubation medium. ApoC-III completely blocked oxidative phosphorylation during oxidation of palmitoyl carnitine by mitochondria, while the respiration rate in metabolic state 4 remained unchanged.

Key Words: *mitochondria; oxidative phosphorylation; apoproteins C; palmitoyl carnitine; calcium*

The structure, physicochemical properties, and metabolism of various plasma lipoproteins are largely determined by their apoproteins. Recent studies showed that blood apolipoproteins take part in the regulation of the most important metabolic processes [5].

Apoproteins C represent a large group of blood apolipoproteins. These low-molecular-weight proteins are surface components of chylomicrons, very low density, and low density lipoproteins (VLDL and LDL). Due to their effect on lipoprotein metabolism they were called "functional apoproteins" [1].

The effect of total apoC fraction on oxidative phosphorylation has been studied previously [5]. ApoC consists of 3 proteins (apoC-I, apoC-II, an apoC-III) with different chemical structure, and therefore the contribution of each component to the regulation of oxidative phosphorylation deserves special attention.

We investigated the effects of each apoC protein on energy parameters of rat liver mitochondria during oxidation of substrates providing reducing equivalents to various sites of the respiratory chain.

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MATERIALS AND METHODS

The study was carried out on 12 male Wistar rats weighing 180-200 g. ApoC-I, apoC-II, and apoC-III were isolated by gel filtration on Sepharose 4B (Pharmacia). Purified apoproteins were stored at 4°C. Mitochondria were isolated from rat liver by differential centrifugation [6] in a medium containing 250 mM sucrose (Serva), 10 mM Tris-HCl (pH 7.4), 1 mM EDTA (both from Reanal), washed, and resuspended in the same medium without EDTA. Protein was measured by Lowry's method [4], the rate of oxygen consumption was evaluated by polarography at 26°C using a platinum electrode with polystyrene coating. Mitochondria were incubated in a medium containing 100 mM sucrose, 50 mM KCl, 20 mM Tris-HCl (pH 7.4), 5 mM KH_2PO_4, 1 mM $MgCl_2$, 5 mM succinate (Serva), and rotenone (Sigma) in a dose of 2 μg/mg protein with and without EGTA.

For investigating the effects of apoC-I, apoC-II, and apoC-III on oxidative phosphorylation, the mitochondria were preincubated with apoproteins at 26°C for 8 min. The following parameters were measured: rates of substrate oxidation in two metabolic states

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TABLE 1. Effects of ApoC-I, ApoC-II, and ApoC-III on Oxidative Phosphorylation in Rat Liver Mitochondria during Succinate Oxidation in EGTA-Free Medium (*M±m*)

Parameter	No apoC (*n*=13)	ApoC-I, 5.7 μg/mg (*n*=6)	ApoC-II, 5.7 μg/ml (*n*=3)	ApoC-III	
				3.0 μg/mg (*n*=6)	5.8 μg/ml (*n*=8)
MS-4	24.0±0.4	24.4±0.4	23.7±0.8	24.5±0.7	24.0±0.6
MS-3	89.9±4.2	88.4±3.8	89.8±6.4	76.3±0.8***	60.6±2.4*
MS_{UNC}	114.1±4.8	116.1±5.1	114.2±11.3	91.3±7.0*	76.5±7.4*
RC	3.8±0.2	3.7±0.2	3.8±0.1	3.1±0.1*	2.5±0.1*
ADP/O	1.67±0.03	1.70±0.01	1.67±0.03	1.55±0.01	1.45±0.04**
V_P	151.6±8.9	151.9±6.9	151.8±11.1	124.6±1.6	90.8±4.6*

Note. Here and in Tables 2 and 3: *$p<0.001$, **$p<0.01$, ***$p<0.05$ *vs.* the control (without apoC). Rates of oxygen consumption in MS-4, MS-3, and MS_{UNC} are presented in ng atomic oxygen/min/mg protein; V_P in nm ADP/min/mg protein.

according to Chance (MS-3, MS-4) [3] and in uncoupled state (MS_{UNC}), phosphorylation rate (V_P), ADP/O coefficient, and respiratory control (RC) according to Chance. Transition into MS-4 was initiated by adding oxidation substrates (10 mM succinate, 5 mM pyruvate+1 mM malate; 25 μM palmitoyl carnitine+1 mM malate, Serva). Transition into MS-3 was induced by adding 150 μM ADP (Reanal). Chlorocarbonylcyanide phenylhydrasone (0.5 μM Sigma) was used as the uncoupling agent.

The results were statistically processed using Student's *t* test.

RESULTS

Incubation of mitochondria in the presence of apoC-I and apoC-II in a concentration of 5.7 μg/mg mitochondrial protein did not change oxidative phosphorylation during succinate oxidation (Table 1). ApoC-III in a dose of 3 μg/mg protein decreased the respiratory rate with the same substrate by 15% in MS-3 and by 20% in MS_{UNC} in comparison with the control. When the dose was increased to 5.8 μg/mg protein, the respiration rate in MS-3 and MS_{UNC} decreased by 32 and 33%, respectively. V_P, ADP/O, and RC also decreased. These data attest to a dose-dependent effect of apoC-III.

Previously we showed that VLDL apolipoproteins modulated oxidative phosphorylation in rat liver mitochondria in EGTA-free medium [2,5]. In the present study addition of Ca ion chelator EGTA to the incubation medium abolished the inhibitory effect of apoC-III on succinate oxidation (Table 2). Therefore, apoC-III constituting about 40% VLDL proteins acts via a Ca^{2+}-dependent mechanism.

The effect of apoC-III was more expressed during mitochondrial oxidation of NAD^+-dependent substrates pyruvate and malate. At a dose of 5.8 μg/mg protein, the respiratory rate in MS-3 and MS_{UNC} was inhibited by 40% and V_P and ADP/O coefficient decreased almost twofold (Table 2).

The inhibitory effect of apoC-III was more pronounced during palmitoyl carnitine oxidation by mitochondria. In a dose of 3.1 μg/mg mitochondrial protein, apoC-III inhibited respiration by 40% in MS-3 and by 61% in MS_{UNC}. In the presence of 5.9 μg/mg

TABLE 2. Effects of ApoC-III on Oxidative Phosphorylation in Rat Liver Mitochondria during Oxidation of Succinate, Pyruvate, and Malate (*M±m*)

Parameter	Succinate oxidation in EGTA-free medium		Pyruvate and malate oxidation in EGTA-free medium	
	No apoC-III (*n*=8)	ApoC-III, 6.9 μg/mg (*n*=4)	No apoC-III (*n*=4)	ApoC-III, 5.8 μg/mg (*n*=4)
MS-4	22.1±0.7	21.3±0.6	14.5±0.5	14.4±0.5
MS-3	93.1±5.4	84.5±1.7	50.7±5.1	29.7±2.7*
MS_{DIS}	119.3±12.2	110.9±7.8	59.0±4.4	35.0±4.5*
RC	4.2±0.2	3.8±0.1	3.5±0.3	2.1±0.2*
ADP/O	1.52±0.05	1.67±0.06	2.11±0.05	1.67±0.18*
V_P	141.0±9.0	138.0±1.8	106.4±10.3	50.1±8.8*

TABLE 3. Effects of ApoC-I and ApoC-III (μg/mg Protein) on Oxidative Phosphorylation in Rat Liver Mitochondria during Oxidation of Palmitoyl Carnitine and Malate in EGTA-Free Medium ($M \pm m$)

Parameter	No apoC-III (n=7)	ApoC-I, 5.6 μg/mg (n=3)	ApoC-III	
			3.1 μg/mg (n=5)	5.9 μg/mg (n=6)
MS-4	17.7±0.8	16.8±0.5	16.9±0.6	16.8±0.9
MS-3	53.1±2.7	57.4±3.1	34.2±2.1***	18.2±1.8*
MS_{UNC}	71.4±1.8	71.8±4.1	27.7±5.0***	16.1±1.6*
RC	3.1±0.3	3.5±0.3	2.0±0.2***	1.1±0.1*
ADP/O	2.26±0.05	2.30±0.09	1.93±0.08**	0
V_P	120.6±7.1	133.2±11.3	66.8±5.4*	0

apoC-III protein, the rate of oxygen consumption decreased by 68% in MS-3 and by 78% in MS_{UNC}. Oxidative phosphorylation was completely inhibited. ADP/O coefficient could not be determined under these conditions (Table 3). ApoC-I did not affect mitochondrial respiration during palmitoyl carnitine oxidation (Table 3).

Hence, only one apoprotein of the apoC family produced a dose-dependent effect on mitochondrial respiration. The mechanism of apoC-III effect on oxidative phosphorylation is not yet clear. At present we investigate adenine nucleotide translocase as a possible target of apoC-III action.

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