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RESPIRATION OF MITOCHONDRIA AND STATE OF PHOSPHORYLATION OF RAT LIVER ADENINE NUCLEOTIDES AFTER REPEATED ADMINISTRATION OF 3-METHYLCHOLANTHRENE AND PHENOBARBITAL

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After four injections of phenobarbital (PB) and 3-methylcholanthrene (MC) in olive oil and a single injection of olive oil into rats the acyl-CoA content in the liver (in % of the control) was 73, 167, and 230 respectively. The liver mitochondria of rats receiving injections of oil and MC were characterized by a decrease in the respiration rate in Chance's 3rd metabolic state, but this was abolished by preincubation with carnitine. The blood ketone body level after injection of PB, MC, and oil was 31, 136, and 342% respectively. The phosphate potential was lowered only after injection of oil, when the ATP concentration in the liver was considerably reduced. The AMP concentration in the liver was doubled after injections of PB and oil. Comparison of the data for induction of microsomal monooxygenases of PB and MC leads to the conclusion that acyl-CoA metabolism proceeds in different directions in the two cases.

KEY WORDS: induction; injection of oil; adenine nucleotides of the liver; respiration of mitochondria; liver acyl-CoA.

Mitochondrial adenine-nucleotide translocase (ANT) controls both the kinetics of the phosphorylating respiration of the mitochondria [1] and the level of the cytoplasmic phosphate potential [8]. It has accordingly been postulated that inhibition of the mitochondrial carrier of ATP and ADP by acyl-derivatives of coenzyme A, especially by palmitoyl-CoA, plays an important role in the mechanisms of regulation of energy metabolism during adaptation to altered metabolic conditions [11].

The object of this investigation was to study the connection between changes in the acyl-CoA concentration and respiration of the mitochondria and the state of phosphorylation of cytoplasmic adenine nucleotides after injection of 3-methylcholanthrene (MC) and phenobarbital (PB) into rats.

EXPERIMENTAL METHOD

Male Wistar rats were given daily injections of PB (10 mg/100 g body weight in 0.9% NaCl solution) and MC (2 mg/100 g body weight in 0.5 ml olive oil) for 4 days. Some animals received injections of olive oil only. Before the experiments the rats were deprived of food for 24 h. Mitochondrial respiration was determined polarographically as described earlier [10]. The concentrations of ATP, ADP, and AMP in the liver were de-

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TABLE 1. Content of Acyl-CoA and CoA-SH in the Liver and Total Blood Ketone Bodies after Injection of PB, MC, and Oil into Rats (M ± m)

Agent	Acyl-CoA	CoA-SH	Acyl-CoA/CoA-SH	Total blood ketone bodies, mg %
	nmoles/g wet weight			
Control	45.9±18 (6)	161±4.7 (6)	0.29	5.3±0.9 (8)
PB	33.6±4.4 (6)	104.7±18 (6)	0.32	1.61±0.64 (9)
MC	76.9±4.4 (6)	123±4.3 (6)	0.63	7.05±4.8 (7)
Oil	105.4±19.4 (6)	115±35.4 (6)	0.92	18.1±5.5 (8)

Legend. Number of rats tested given in parentheses.

TABLE 2. Effect of Carnitine on ATP-Stimulated Respiration of Liver Mitochondria after Injection of PB, MC, and Oil into Rats

Agent	Substances added	Mitochondrial respiration rate in different metabolic states (MS), after Chance, in nanoatoms O_2/min/mg protein					
		MS_4	MS_3	MS_4	MS_3	MS_4	MS_3
Control	Glutamate + malate	16	56	17	58	17	63
	Glutamate + CCCP	16	64	—	—	—	—
	Glutamate + Carnitine	16	58	17	56	16	64
	Succinate + rotenone	20	105	25	119	28	128
	Succinate + CCCP	20	128	—	—	—	—
	Succinate + Carnitine	21	109	25	120	27	120
PB	Glutamate + malate	12	53	12	53	13	63
	Glutamate + CCCP	12	63	—	—	—	—
	Glutamate + Carnitine	12	64	13	63	13	64
	Succinate + rotenone	32	110	32	100	34	100
	Succinate + CCCP	32	111	—	—	—	—
	Succinate + Carnitine	30	110	29	100	29	110
MC	Glutamate + malate	13	44	13	52	13	52
	Glutamate + CCCP	12	65	—	—	—	—
	Glutamate + Carnitine	13	53	12	60	12	65
	Succinate + rotenone	21	86	22	98	—	—
	Succinate + CCCP	21	118	—	—	—	—
	Succinate + Carnitine	21	95	21	114	—	—
Oil	Glutamate + malate	13	42	17	47	19	53
	Glutamate + CCCP	12	82	—	—	—	—
	Glutamate + Carnitine	12	50	19	55	22	77
	Succinate + rotenone	23	55	17	47	19	53
	Succinate + CCCP	23	131	—	—	—	—
	Succinate + Carnitine	25	77	35	100	48	128

*CCCP) chlorocarbonyl-cyanide phenylhydrazone. Maximally uncoupled respiration obtained by titration with CCCP.

TABLE 3. State of the Rat Liver Adenine Nucleotide System after Injection of PB, MC, and Oil (M ± m)

Agent	ATP	ADP	AMP	Total adenine nucleotides	P_i	ATP/ ADP · P_i	ATP + 0.5 ADP / ATP + ADP + AMP
	nmoles/g wet weight of liver						
Control	1842±209	1250±138	480±113	3572	3883±486	0,38	0,69
PB	1787±92	1140±153	1136±171	4063	3523±336	0,44	0,58
MC	1799±380	1277±275	839±151	3815	2867±372	0,46	0,61
Oil	1249±189	1160±129	1098±154	3507	5326±545	0,20	0,52

Legend. Mean results of six experiments given.

termined by an enzymic method [9] with the Specol (East Germany) spectrofluorometer. Total blood ketone bodies were determined by the Todorov's method [2], the concentrations of acyl-CoA and CoA-SH in the liver by the method of Tubbs and Garland [12], mitochondrial protein by the biuret method [7], and inorganic phosphorus (P_i) by the method of Berenblum and Chain [4].

EXPERIMENTAL RESULTS AND DISCUSSION

To study the effect of acyl-CoA on ADP-stimulated mitochondrial respiration and the state of phosphorylation of adenine nucleotides in the liver, the models of induction of microsomal monooxygenases by MC and PB were chosen on the basis of the results showing that, by contrast with MC, injection of PB is followed by an increase in the quantity of membranes of the endoplasmic reticulum, including their phospholipid component [6]. It might be expected that after injection of PB, MC in oil, and of oil alone, difference in the direction of acyl-CoA metabolism and in the levels of energy expenditure and the state of oxidative phosphorylation in the liver mitochondria would be observed.

After injection of oil alone the concentration of acyl-CoA in the liver increased by 2.3 times, after injection of MC it increased by 1.7 times, but after injection of PB there was actually a small decrease in the acyl-CoA concentration (Table 1). The differences between the series of experiments as regards the levels of esterified and free coenzyme A are displayed more clearly in Table 1 as acyl-CoA/CoA-SH ratios.

The mitochondrial respiration rates are shown in Table 2 as consecutive changes from Chance's fourth metabolic state into the third state [5]; they show that, compared with the theoretically maximal rate of uncoupled respiration, inhibition of mitochondrial respiration was observed in response to addition of ADP in the animals after injection of oil and, to a rather lesser degree, after injection of MC. Inhibition of ADP-stimulated respiration of the liver mitochondria of these animals was abolished by carnitine. As was stated previously [10], the ability of carnitine to abolish inhibition of ADP-stimulated respiration is evidence of a disturbance of the function of the adenine nucleotide carrier, acyl-CoA.

The degree of inhibition of ADP-stimulated mitochondrial respiration thus corresponded to the degree of increase in the acyl-CoA concentration in the liver of the rats after injection of oil and MC. Also in agreement with these effects was an increase in the total blood level of ketone bodies (Table 1), the synthesis of which in the liver mitochondria depends on the level of reduction of mitochondrial pyridine nucleotides during β-oxidation [11].

Data for the content of ATP, ADP, AMP, and P_i in the rats' livers and the corresponding values of the phosphate potentials (ATP/ADP · P_i) and Atkinson's potential (ATP + 0.5 ADP/ATP + ADP + AMP) which, according to its author is a measure of the "energy charge of the adenyl system" [3], are given in Table 3. It will be clear from Table 3 that only after injection of oil was there a marked decrease in the ATP concentration and this, together with the increased P_i concentration, almost halved the value of the phosphate potential. After injection of MC the level of the phosphate potential was actually higher than in the control, as a result of the decrease in the P_i concentration. After injection of PB and oil the AMP level was doubled, in agreement with the hypothesis that under these conditions activation of fatty acids is intensified. The absence of any increase in the acyl-CoA concentration in the rats' livers after injection of PB in the presence of a raised AMP level, by contrast with the animals receiving oil alone, can be regarded as an indirect indication of the intensified utilization of the acyl-CoA formed during synthesis of the membrane phospholipids of the endoplasmic reticulum, the quantity of which is higher than normal during induction of microsomal monooxygenases of PB [6].

The consistency of the changes in Atkinson's potential and the differences in the values of the phosphate potential after injection of PB and oil were attributed to the fact that the phosphate potential reflects the state of the liver adenyl system as it depends on the state of oxidative phosphorylation [8], whereas Atkinson's potential reflects ATP-utilizing processes bound with AMP generation (for example, the synthesis of acyl-CoA). The effect of MC on the acyl-CoA content, on the state of the adenine nucleotide system, and on the other indices cannot be given a single interpretation, for it follows from the results shown in Tables 1-3 that MC in oil abolishes many of the effects of the oil itself.

On the whole the results described confirm the earlier hypothesis that the inhibitory effects of acyl-CoA on the transfer of adenine nucleotides through the inner membrane of the mitochondria may perform a regulatory role during adaptation of energy metabolism to different metabolic situations.

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