EFFECT OF BINDING OF PALMITOYL-CoA WITH ADENINE-NUCLEOTIDE TRANSLOCASE ON MITOCHONDRIAL ENERGIZATION

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During inhibition of oxidative phosphorylation by oligomycin, palmitoyl-CoA (p-CoA) reduces the velocity of energy-dependent reduction of acetoacetate and the Ca^{++}-capacity of the mitochondria in medium with phosphate. Energy-independent osmotic swelling of the mitochondria in medium with NH_4NO_3, which depends on the proton conductance of the inner membrane, is inhibited by ADP and accelerated by p-CoA. All effects of p-CoA are abolished by carnitine and competitively by ADP. It is concluded that the lowering of the energization level by p-CoA is connected with increased permeability of the inner membrane for H^+ as a result of binding of the inhibitor with adenine-nucleotide translocase.

KEY WORDS: energization; palmitoyl-CoA; adenine-nucleotide translocase; liver mitochondria.

The importance of the study of the metabolic consequences of interaction between palmitoyl-CoA (p-CoA) and the mitochondrial membrane is due to the increased content of acyl-CoA in the liver in some physiological and pathological states (starvation, diabetes) [7, 9]. Investigations have shown that acyl-CoA and, in particular, p-CoA, interacts specifically with mitochondrial adenine-nucleotide translocase (ANT) [1, 6, 10], resembling in its action atractyloside (AT) and carboxyatractyloside (CAT). However, the state of other mitochondrial functions when modified by interaction between acyl-CoA and ANT, with the exception of inhibition of adenyl-nucleotide (AN) transport, has not been adequately studied.

The object of this investigation was to study the effect of p-CoA on energy-dependent functions of the mitochondria (MCH).

EXPERIMENTAL METHOD

MCH were isolated from the liver of male Wistar rats by the method described in [12]. The 3-hydroxybutyrate content in MCH was determined by an enzymic method [14]. Incubation and preparation of samples for spectrophotometry were carried out by Azzone's method [4]. The uptake of Ca^{++} by MCH in the presence of phosphate was determined from the change in pH of the medium during addition of equal quantities of $CaCl_2$ at equal time intervals until the onset of deenergization of MCH. Osmotic changes in the volume of MCH were recorded on the Hitachi-556 spectrophotometer at 546 nm. All the experiments were carried out after preincubation for 2 min with oligomycin to inhibit oxidative phosphorylation. The protein content in MCH was determined by the biuret method [8].

EXPERIMENTAL RESULTS

Reduction of exogenous acetoacetate by MCH to 3-hydroxybutyrate during reverse electron transport along the respiratory chain depends on energization of MCH [4]. It was shown in Fig. 1 that in the presence of p-CoA in the proportion of 3 nmoles/mg protein the velocity of acetoacetate reduction was reduced about by half compared with the control. On the addition of 20 μM ADP, on the other hand, the rate of reduction of acetoacetate was doubled. Carnitine largely abolished the effect of p-CoA, and the combined addition of carnitine and ADP completely abolished the inhibitory effect of p-CoA on energy-dependent reduction of acetoacetate. Atractyloside had a similar action to p-CoA on this function of MCH.

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TABLE 1. Effect of Palmitoyl-CoA, ADP, and Carnitine on Ca^{++}-Capacity of MCH in Medium with Phosphate

Additives	Ca^{++} uptake, nmoles/mg protein
Control	121
ADP (50 μM)	250
p-CoA, nmoles/mg protein	
2	120
4	81.2
6	59
CAT	58
CAT+ADP (200 μM)	55
p-CoA (6 nmoles/mg protein)+ADP (50 μM)	88.5
p-CoA (6 nmoles/mg protein)+ADP (200 μM)	119
p-CoA (4 nmoles/mg protein)+ carnitine	102.5
p-CoA (6 nmoles/mg protein)+ carnitine	65
p-CoA (6 nmoles/mg protein)+ carnitine+ADP (50 μM)	152

Legend. Incubation conditions: sucrose 50 mM, KCl 100 mM, glycyl-glycine 3.3 mM, succinate 5 mM, KH_2PO_4 1 mM, pH 7.4, rotenone 1 μg/mg protein, oligomycin 3 μg/mg protein, carnitine 3.5 mM, CAT 5 μm, protein 1.16 mg ml incubation medium. Volume of cuvette 6 ml. Ca^{++} added in doses of 50 μmoles/-liter (final concentration in cuvette).

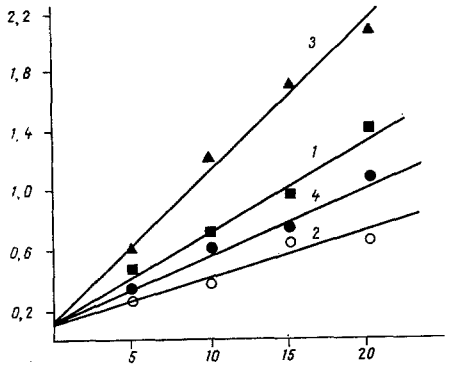

Fig. 1. Effect of palmitoyl-CoA, ADP, and carnitine on energy-dependent formation of 3-hydroxybutyrate from acetoacetate by mitochondria. Conditions of incubation: sucrose 100 mM, KCl 50 mM, glycyl-glycine 20 mM, succinate 10 mM, pH 7.4, oligomycin 3 μg/mg protein, acetoacetate 8 mM, protein 3 mg/ml incubation medium. 1) Control; 2) p-CoA, nmoles/mg protein; 3) 20 μM ADP; 4) 1.5 mM carnitine+pCoA, 3 nmoles/mg protein. Abscissa, time (in min); ordinate, concentration of 3-hydroxybutyrate (in mM).

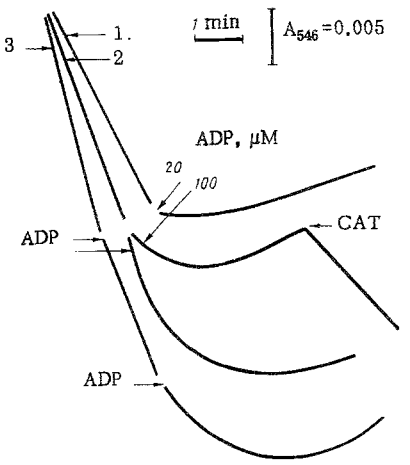

Fig. 2. Effect of palmitoyl-CoA and ADP on passive osmotic swelling of MCH in medium with NH_4NO_3. Conditions of incubation: NH_4NO_3 100 mM, glycyl-glycine 5 mM, pH 7.4, rotenone 1 μg/mg protein, oligomycin 3 μg/mg protein, carboxyatractyloside 5 M, protein 1 mg, volume 3 ml. 1) Control; 2) p-CoA, 2.2 nmoles/-mg protein; 3) p-CoA, 3.3 nmoles/mg protein, ADP added in doses of 20 μM in course of swelling. A_{546}) optical density.

The quantity of Ca^{++} taken up by MCH in the presence of inorganic phosphate is also an indicator of mitochondrial energization [3]. The results of one typical experiment to study the effect of p-CoA and ADP on the Ca^{++}-capacity of MCH during inhibition of oxidative phosphorylation are given in Table 1. It can be seen that p-CoA reduced the quantity of Ca^{++} taken up by MCH without changing the rate of uptake (not shown). ADP, on the other hand, considerably increased the Ca^{++}-capacity of MCH under these conditions. On the addition of increasing concentrations of ADP in the presence of p-CoA, the ever-increasing reversal of the inhibitory effect of p-CoA on Ca^{++} uptake by MCH was observed. This corresponds to the competitive character of relations between p-CoA, ADP, and the specific binding points of these ligands to ANT [1, 10]. Carnitine also reverses the effect of p-CoA, but in high concentrations of p-CoA the reversal was incomplete. Just as in the case of inhibition of reduction of acetoacetate by p-CoA, carnitine and ADP additively abolished the inhibition of Ca^{++} uptake by MCH caused by p-CoA.

To test the hypothesis that p-CoA affects energization of MCH by modifying the permeability of the inner membrane for ions, passive swelling of MCH in medium with NH_4NO_3 was studied. The kinetics of swelling in NH_4NO_3 medium is known [5] to depend on permeability of the membrane for H^+. Starting with a concentration of 5 μM, ADP inhibited the swelling of MCH and maximal inhibition was observed in the presence of 20 μM ADP. p-CoA accelerated the swelling of MCH in NH_4NO_3 medium compared with the control. The effect of p-CoA was abolished by carnitine and competitively by ADP (Fig. 2). Atractyloside had a similar effect to ADP on swelling of MCH in NH_4NO_3 medium.

The results given in Fig. 1 and Table 1 show that ADP in low concentrations increases energization of MCH. On the basis of features of the nonphosphorylating effect of ADP on energy-dependent fractions of MCH listed below it can be postulated that ADP exerts its effect through specific interaction with ANT. Half the maximal effect on the Ca^{++}-capacity of MCH and on energy-dependent reduction of acetoacetate was observed with 5 μM ADP, and the maximal effect with 20 μM ADP, i.e., in concentrations corresponding to half-maximal and maximal binding of nucleotide with carrier [13]. The effects of ADP are competitively abolished by atractyloside and p-CoA and noncompetitively by CAT in the presence of low concentrations of the inhibitors, when their nonspecific binding can be ruled out. The effect of ADP on energization of MCH (Fig. 2) can be explained by the ability of this nucleotide to inhibit proton conductance of the inner membrane of MCH. We concluded that ADP exerts its action on proton conductance of the inner membrane and, through this mechanism, on energization of MCH by means of allosteric modification of the conformation of ANT. The ability of ADP and inhibitors of AN transport to modify the conformation of ANT was demonstrated previously [2].

In the light of these observations the action of p-CoA on the energy-dependent functions of MCH and on the proton conductance of their inner membrane can be satisfactorily explained. p-CoA is known to inhibit AN transport specifically, like atractyloside [1, 8]. The inhibition constant is very low, namely 0.5 μM, and the action of p-CoA is competitive with respect to ADP and ATP. Carnitine abolishes the effect of p-CoA, since the effective concentration of p-CoA is lowered as a result of the activity of carnitine-palmitoyl transferase, and the palmitoyl carnitine formed had no effect on ANT [11]. The ability of ADP and carnitine to abolish the effect of p-CoA additively is explained by the fact that carnitine lowers the p-CoA concentration in the membrane, and ADP under these conditions competes more effectively with p-CoA for the binding sites on ANT.

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LOCALIZATION OF NERVE-SPECIFIC PROTEIN ANTIGENS ON THE SURFACE MEMBRANE OF NEURONS AND GLIAL CELLS OF Helix pomatia

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The existence of cross protein antigens common to several species of invertebrates and vertebrates on the membrane of neurons and glial cells of Helix pomatia was demonstrated in vitro by Coons' immunofluorescence method. The presence of nerve-specific protein S-100 on the membrane of these cells was established. The antigenic heterogeneity of membranes of a population of neurons also was observed. Differences were found in the concentrations of antigens on the somatic and axon membranes. The character of distribution of specific fluorescence indicates possible qualitative and (or) quantitative differences in the content of nerve-specific proteins in different areas of the neuron membrane.

KEY WORDS: brain-specific antigen; neurons of invertebrates; immunofluorescence.

The existence of a class of protein antigens specific for nerve tissue can now be accepted as proven. It is considered that these proteins are responsible for conducting and generating the action potential and for synaptic transmission, participate in mechanisms of memory and learning, and so on [5]. Since many of these functions of the nervous system are connected in some way or other with the activity of the neuron mem-

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