RELATIONS BETWEEN FATTY ACID ACTIVATION AND ADENINE NUCLEOTIDE TRANSLOCASE SYSTEMS IN MITOCHONDRIA

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Experiments with freshly isolated rat liver mitochondria and mitochondria aged by two different methods showed that the activity of the mitochondrial fatty acid activation system can lead to inhibition of adenine nucleotide (AN) transport through the inner mitochondrial membrane. Inhibition of AN translocase was abolished by preincubating the mitochondria with carnitine. A special feature distinguishing inhibition of AN translocase by aged mitochondria is that these mitochondrial membranes contain fatty acids in sufficient concentration to cause inhibition on the addition of CoA and ATP. The results suggest a role for acyl-CoA formed by mitochondrial acyl-CoA synthetase in the regulation of AN transport in the mitochondrion.
KEY WORDS: liver mitochondria; fatty acid activation system; adenine nucleotide translocase; carnitine.

The view that fatty acids with a long carbon chain can affect the transport of adenine nucleotides (AN) in mitochondria was first formulated in papers [14, 15] which showed inhibition of atracetyloside-sensitive binding and metabolism of ADP and ATP in mitochondria and submitochondrial particles on the addition of oleate. Compared with the fatty acid, its activated form (acyl-CoA) has a more marked effect on AN transport [8], and this effect can be found in certain physiological states of the organism characterized by an increased intracellular acyl-CoA concentration [9, 10]. The enzyme acyl-CoA synthetase, which converts long-chain fatty acid into acyl-CoA, is known to be present in the membranes of the endoplasmic reticulum and in the outer mitochondrial membrane [12].

The object of this investigation was to study inhibition of AN translocase by palmitoyl-CoA, formed by the mitochondrial fatty acid activation system.

EXPERIMENTAL METHOD

Male Wistar rats, starved for 12 h before sacrifice, were used to obtain mitochondria. The mitochondria were isolated from the liver by Weinbach's method [13]. Respiration of the mitochondria was determined polarographically with a platinum electrode of semicovered type. The incubation conditions are given in the captions to Figs. 1-3. The content of mitochondrial protein was determined by the biuret method [4].

EXPERIMENTAL RESULTS AND DISCUSSION

Activation of long-chain fatty acids by the enzyme acyl-CoA synthetase, located in the outer mitochondrial membrane, is an important stage in the mechanisms of penetration of fatty acids to the site of their oxidation in mitochondria. Inhibition of AN transport in mitochondria, known to take place on the addition of exogenous palmitoyl-CoA [8], does not explain the relations of the mitochondrial fatty acid activation system and AN translocase. It was therefore decided to study the effect of activation of palmitate on AN transport in rat liver mitochondria.

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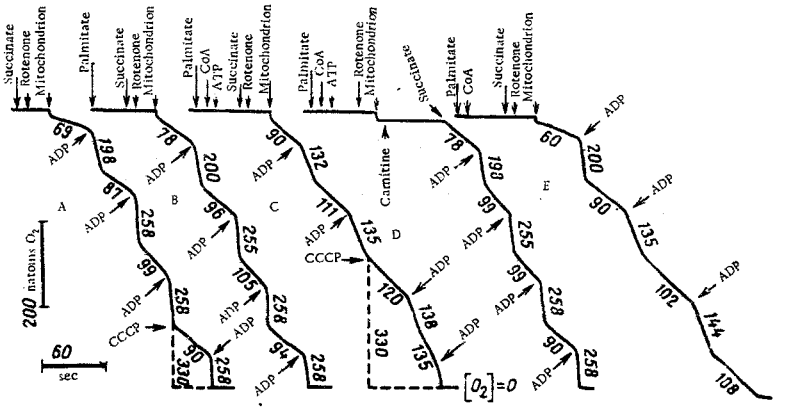

Fig. 1. Effect of activation of palmitate on respiration of freshly isolated mitochondria. Incubation medium (in mM): KCl 125, Tris–HCl buffer, pH 7.4, 20, KH_2PO_4 5, $MgCl_2$ 5. Mitochondrial protein 3 mg. Reagents added: succinate 5 mM, ADP 150 μM, chlorocarbonylcyanide phenylhydrazone (CCCP) $5 \cdot 10^{-7}$M, rotenone 1 μg to 1 mg protein, palmitate 3 μM, CoA 30 μM, carnitine 1 mM. Volume of cuvette 1.35 ml. A) Control; B) palmitate; C) palmitate + CoA + ATP; D) palmitate + CoA + ATP + carnitine; E) palmitate + CoA.

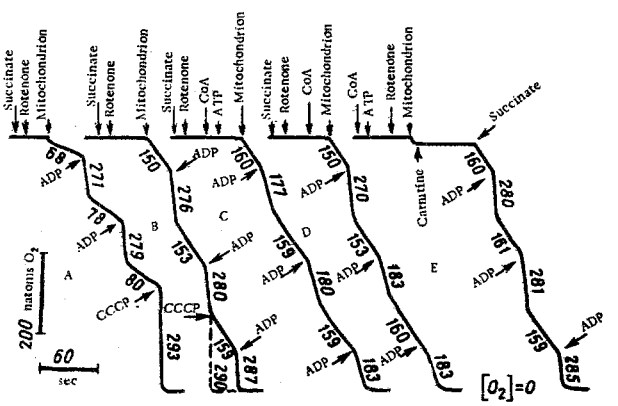

Fig. 2

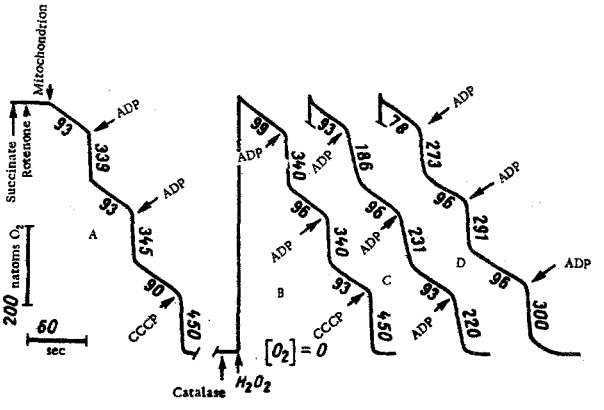

Fig. 3

Fig. 2. Inhibition of ADP-stimulated succinate oxidation by aged mitochondria in the presence of CoA and ATP. Aging of mitochondria for 5 h at 5°C. Conditions of incubation as in Fig. 1. A) Control; B) aging; C) CoA + ATP; D) CoA; E) CoA + ATP + carnitine.

Fig. 3. Respiration of mitochondria aged in oxygen-free medium in presence of CoA and ATP. Aging time 15 min. Return to aerobiosis achieved by addition of catalase (2 μg/ml) and hydrogen peroxide ($5 \cdot 10^{-3}$ ml of 1% solution). Conditions of incubation as in Fig. 1. A) Control; B) aging; C) CoA + ATP; D) CoA + ATP + carnitine.

Experiments to record respiration of freshly isolated mitochondria (Fig. 1A, B) showed that palmitate in a concentration of 3 mM did not affect ADP-stimulated oxidation of succinate by mitochondria. In all experiments rotenone was added to the incubation medium to prevent oxidation of palmitate by the mitochondria. On the addition of palmitate, coenzyme A, and ATP to this medium the respiration rate of the succinate-oxidizing mitochondria was reduced in state 3 on average by 34% compared with the control, whereas the rate of uncoupled respiration was unchanged. This decrease in the respiration rate of the mitochondria was caused by inhibition of AN transport through the inner mitochondrial membrane by palmitoyl-CoA formed as a result of the activity of mitochondrial acyl-CoA synthetase, for carnitine, if added in a concentration of 1 mM, restored the maximal velocity of succinate oxidation in state 3 (Fig. 1F), by liberating the AN carrier from the inhibitor. On the addition of palmitate and CoA only to the mitochondria (Fig. 1E), inhibition of mitochondrial respiration in state 3 was manifested as a decrease in the response only to the second addition of ADP. Judging from the

manifestation of the inhibitory effect of palmitoyl-CoA it must be considered that in this case fatty acid activation took place with the utilization of ATP leaving the mitochondria in exchange for the first addition of ADP.

It was shown previously that aging of mitochondria in vitro is accompanied by a decrease in the content of mitochondrial phospholipids and a stoichiometric increase in the concentration of free fatty acids (palmitic, stearic, oleic, linoleic, and arachidic) as a result of activation of endogenous phospholipases [2, 3]. Accordingly the ADP-stimulated oxidation of succinate by aging mitochondria in the presence of CoA and ATP or of CoA alone was studied. As Fig. 2 shows, the addition of CoA and ATP to such mitochondria reduced the rate of respiration of the mitochondria in state 3, during oxidation of succinate, which was presented by the addition of carnitine. Considering the absence of this effect on freshly isolated mitochondria, it can be concluded that the substrate for the activating system under these conditions must be fatty acids formed during hydrolysis of mitochondrial phospholipids.

Experimental data on inhibition of AN translocase by acyl-CoA in mitochondria isolated from ischemic heart tissue have been obtained [11]. A convenient model for studying the effect of ischemic states on the mitochondrion is anaerobic aging of mitochondria in vitro. It was shown previously that, besides other changes, anaerobiosis also causes a decrease in respiratory control and the respiration rate in state 3 in mitochondria, and that these can largely be reversed by the addition of bovine serum albumin [6]. The effect of small periods of anaerobiosis (15-20 min) on respiration of the mitochondria was investigated. It will be clear from Fig. 3 that anaerobic aging for 15 min at 25°C caused practically no change in the coupling of oxidation and phosphorylation in the mitochondria. Meanwhile, on the addition of CoA and ATP to the incubation medium there was a marked decrease in respiration in state 3, which can be attributed to the action of the newly formed acyl-CoA on AN translocase.

The study of factors influencing AN translocase activity is of considerable interest, for highly specific transport of ATP and ADT in mitochondria is the limiting state that determines the overall rate of phosphorylation of exogenous ADP or hydrolysis of ATP [5, 7]. The results of the present experiments indicate that the working of the mitochondrial fatty acid activation system in vitro may lead to inhibition of AN transport through the inner mitochondrial membrane. In recently isolated and aged mitochondria, and also in mitochondria aged in an oxygen-free medium, no difference was found as regards fatty acid activation, manifested as the inhibitory effect of acyl-CoA on AN transport in all the cases examined. A feature which distinguishes the inhibition of AN translocase in mitochondria aged by the two different methods is the presence of fatty acids in these mitochondria in a sufficient concentration to cause inhibition on the addition of CoA and ATP. In ischemic tissue cells the content of free fatty acids and inorganic phosphate is considerably increased, and in high concentrations the latter can cause a high-amplitude swelling of the mitochondria with the liberation of mitochondrial CoA [1, 6].

The model situation examined in vitro in these experiments, with inhibition of the adenine nucleotide translocase of mitochondria aged in an anaerobic medium, may thus reflect real changes in mitochondrial metabolism in a tissue or organ in an ischemic state.

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