A POSSIBLE ROLE OF ADENINE-NUCLEOTIDE TRANSPORT IN THE REGULATION OF RESPIRATION OF RAT LIVER MITOCHONDRIA

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A study of respiration of the liver mitochondria of rats after 48 h of starvation revealed a fall of 20% in the rate of ADP-stimulated substrate oxidation compared with the rate of uncoupled respiration. This effect was abolished by preincubation of the mitochondria with carnitine. In the case of liver mitochondria from satiated rats there was a marked decrease in the rate of respiration both in state 3 and in state 4, which was not abolished by carnitine. Preincubation of these mitochondria with α-ketoglutarate led to an increase in the rate of respiration in states 3 and 4 during succinate oxidation. The results suggest the existence of at least two methods of regulation of adenine-nucleotide transport in the mitochondrion depending on the metabolic state of the organism: 1) inhibition of adenine-nucleotide translocase by cytoplasmic acyl-CoA; 2) control of the state of the endogenous adenine-nucleotide reserves in the mitochondrion.

KEY WORDS: adenine-nucleotide transport; liver mitochondria; respiration; starvation.

It has recently become clear that the transport of ADP and ATP through the inner mitochondrial membrane with the aid of adenine-nucleotide translocase (ATase) as the carrier is an important stage in the general process of oxidative phosphorylation [2-4]. It is a fact of great interest that acyl derivatives of coenzyme A, such as palmitoyl-CoA, are competitive inhibitors of ATase [8, 12]. The effect of acyl-CoA on adenine nucleotide transport may perhaps play an important role in the regulation of mitochondrial functions, for their concentration varies with the metabolic state of the organism [5, 7, 11]. However, the inhibition of adenine-nucleotide transport through the inner mitochondrial membrane by acyl derivatives of CoA has been proven only for a system in vitro, and some workers deny the physiological role of this effect [8].

The object of this investigation was to study the possible physiological role of acyl derivatives of coenzyme A in the regulation of ADP-dependent mitochondrial respiration depending on the metabolic state of the organism as reflected in starvation for 48 h and satiety.

EXPERIMENTAL METHOD

Male Wistar rats kept on a balanced diet were deprived of food for 48 h before sacrifice. In experiments on satiated animals the rats were decaptiated 1 h after feeding. Liver mitochondria were isolated by Weinbach's method [14]. Mitochondrial respiration was determined polarographically with a platinum electrode of the semi-open type. The incubation conditions are given in the captions to the figures. Protein was determined by the biuret method [1].

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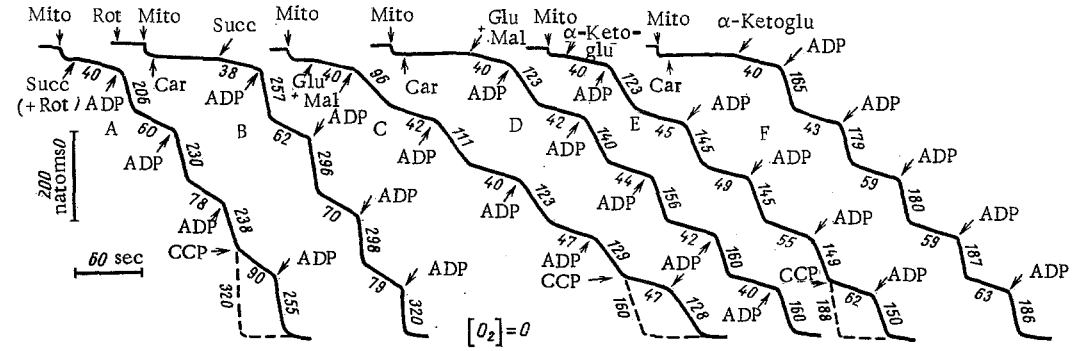

Fig. 1. Respiration of liver mitochondria of fasting rats. Medium: KCl 125 mM, tris-HCl 20 mM, pH 7.4, KH_2PO_4 5 mM, $MgCl_2$ 0.5 mM. Mitochondrial protein (Mito) 2.5 mg. Substances added: succinate (Succ) 5 mM, glutamate (Glu) with malate (Mal) 5 and 1 mM respectively, α-ketoglutarate (α-Ketoglu) 5 mM, ADP 100 mM, chlorocarbonylcyanide-phenylhydrazone (CCP) $5 \cdot 10^{-7}$ M, carnitine (Car) 1.25 mM, rotenone (Rot) 3 mM.

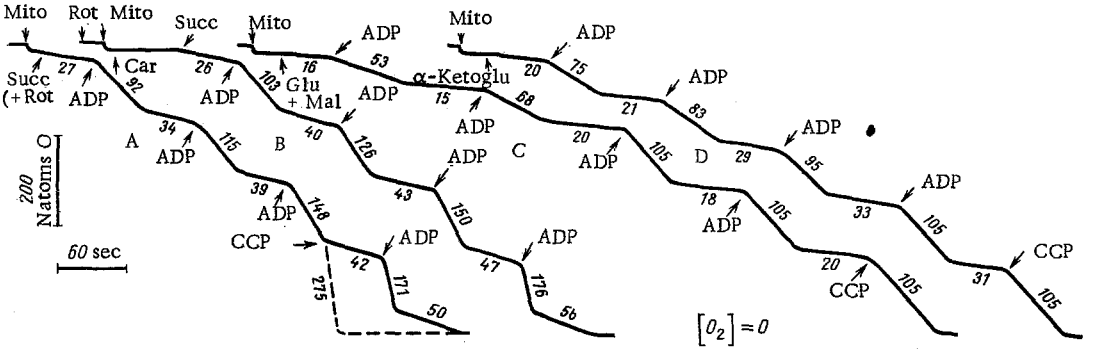

Fig. 2. Respiration of liver mitochondria of satiated rats (conditions of incubation as in Fig. 1).

EXPERIMENTAL RESULTS AND DISCUSSION

Mitochondria from the liver of the starved rats had a rate of respiration in state 3 on the average 20% lower than the rate of uncoupled respiration (Fig. 1A, C, E). This fall was observed both during oxidation of succinate by the mitochondria and during oxidation of NAD-dependent substrates: glutamate with malate, and α-ketoglutarate. Considering the increased concept of acyl-CoA in the liver cells during starvation [7, 11], the observed fall in the rate of ADP-stimulated respiration in the liver mitochondria of the fasting rats was considered to be due to binding of acyl-CoA with ATase. As Fig. 1B, D, F shows, preincubation of the mitochondria with 1 mM carnitine led to an increase in the rate of respiration after the addition of ADP which, in this case, was almost indistinguishable from the rate of respiration after uncoupling. Abolition of the inhibition was probably due to removal of acyl-CoA from the carrier as a result of the formation of acyl-carnitine and its transport through the inner mitochondrial membrane. It is known that up to 50% of the intracellular activity of palmitoyl-carnitine transferase is localized in this membrane [13]. Inhibition of adenine nucleotide transport revealed by these experiments on fasting animals can evidently explain the increase in the intramitochondrial phosphate potential under these same conditions [7].

Experiments with mitochondria isolated from the liver of the satiated animals showed a decrease in the rates of respiration in both state 3 and state 4 compared with respiration of mitochondria from the liver of the fasting rats (Fig. 2A, C, D). In this case preincubation of the mitochondria with carnitine had no effect on the rate of ADP-dependent respiration (Fig. 2B). It was also found that α-ketoglutarate is oxidized dized in states 3 and 4 at higher rates than glutamate with malate. The rate of phosphorylation of exogenous ADP is known to be directly proportional to the ATP concentration inside the mitochondria [2]. It was accordingly postulated that the decrease in the rate of respiration of these mitochondria in state 3 is linked with a decrease in the metabolic reserves of adenine nucleotides (ATP + ADP) and an increase in the concentration of AMP in the matrix. This state of the intramitochondrial adenine nucleotides could be a metabolic distinguishing feature of the liver mitochondria of the satiated rats due to activation of the free fatty acids inside the mitochondria. The rate of oxidation of exogenous free fatty acids by the liver mitochondria can

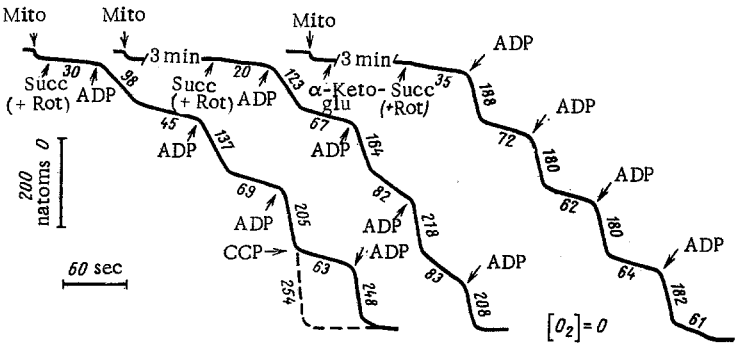

Fig. 3. Effect of preincubation with α-ketoglutarate on ADP-dependent oxidation of succinate by liver mitochondria of satiated rats (conditions of incubation as in Fig. 1).

be very high [6]. Some interesting results in this connection have been obtained by Rossi et al. [10], who showed that a change in the intramitochondrial adenine nucleotides in the direction of an increase in the AMP concentration during oxidation of oleate is prevented by simultaneous oxidation of α-ketoglutarate.

The results of experiments to study the effect of preliminary incubation with α-ketoglutarate on ADP-dependent oxidation of succinate by the liver mitochondria of satiated rats are given in Fig. 3. As a result of preincubation with α-ketoglutarate the rate of respiration of the mitochondria in states 3 and 4 clearly was considerably increased compared with the control. These results can be explained by an increase in the metabolic reserves of adenine nucleotides within the mitochondria as a result of the conversion of AMP into ATP in the presence of substrate phosphorylation [10].

Highly specific transport of ATP and ADP through the inner mitochondrial membrane is the limiting stage that determines the overall rate of phosphorylation of exogenous ADP or hydrolysis of ATP [2, 4]. The results described in this paper indicate that adenine-nucleotide transport through the inner mitochondrial membrane depends on the metabolic state of the organism and can be controlled in at least two ways. In fasting animals ATase is controlled by the inhibitory effect of acyl-derivatives of coenzyme A. In satiated animals the rate of transport of adenine nucleotides is controlled by the size of their metabolic reserves.

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