Carboxyatractylate-sensitive uncoupling in liver mitochondria from ground squirrels during hibernation and arousal

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Energy coupling parameters of liver mitochondria from hibernating and arousing ground squirrels have been studied. In the oligomycin-treated mitochondria, carboxyatractylate, an inhibitor of the ATP/ADP-antiporter, is shown to decrease the respiration rate, to increase the membrane potential and to lower the rate of the membrane-potential discharge after the addition of cyanide to liver mitochondria from hibernating and arousing animals. BSA effectively substitutes for carboxyactactylate so that carboxyactactylate, added after BSA, has no effect. In mitochondria from hibernating animals, the maximal respiration rate in the presence of DNP and the rate of the membrane potential discharge in its absence are much lower than in those from arousing animals. It has been concluded that upon arousal of the animals from hibernation, the uncouling of oxidative phosphorylation, mediated by free fatty acids and ATP/ADP-antiporter, parallels the respiratory chain activation.

Hibernation; Uncoupling; Fatty acid; ATP/ADP-antiporter

1. INTRODUCTION

In 1965 endogenous free fatty acids were found in this group to be mediators of a thermoregulatory uncoupling of oxidative phosphorylation, which developed in skeletal muscle mitochondria in response to the short-term cold exposure of mouse [1]. Recently, we have shown that carboxyatractylate, an inhibitor of the ATP/ADP-antiporter, and its substrate, ADP, suppress the uncoupling action of low concentrations of the added FFA in the presence of oligomycin. According to the above and some other data, the ATP/ADP-antiporter must be involved in the uncoupling of oxidative phosphorylation by the added FFA [2,3]. It might be of interest to learn how this mechanism functions upon activation of thermogenesis under physiological conditions.

In this paper, liver mitochondria of ground squirrels during the arousal from hibernation have been studied. During arousal, mitochondrial respiratory chain activity essentially increases [4] and uncoupling of oxidative phosphorylation develops in liver [5]. These processes are concomitant to the increase in noradrenaline [6] and glucagon [7] production which enhances lipolysis. It has been established that during arousal the level of FFAs in blood plasma increases [8–10].

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Abbreviations: BSA, bovine serum albumin; CAtr, carboxyatractylate; DNP, 2,4-*p*-dinitrophenol; FFA, free fatty acid; TPP^+, tetraphenyl phosphonium; Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)

2. MATERIALS AND METHODS

In this work, carried out in winter, we studied liver mitochondria isolated from ground squirrels *Citellus undulatus* during hibernation, arousal and two weeks after arousal. The isolation medium contained 0.3 M sucrose, 0.5 mM EGTA, 5 mM Hepes, pH 7.4. Respiration was monitored by the polarographic method. Membrane potential was recorded by a TPP^+-sensitive electrode, as described earlier [3]. Mitochondria were suspended in the isolation medium without EGTA (the mitochondrial protein concentration was 70–90 $mg \cdot ml^{-1}$). Mitochondria were incubated at 37°C in a medium containing 250 mM sucrose, 0.5 mM EGTA, 5 mM Hepes, pH 7.4. The oxidation substrates, 4 mM glutamate and 2.5 mM malate were used.

The respiratory activity of mitochondria was estimated by measuring the rate of uncoupled respiration in the presence of 40 μM DNP, which was found to induce maximal stimulation of the respiration rate. The ion conductance of the inner mitochondrial membrane was estimated by the rate of the membrane potential decrease after the addition of 2 mM NaCN to mitochondria oxidizing glutamate and malate in the presence of oligomycin [11,12].

Oligomycin, carboxyactactylate, fatty acid-free BSA, EGTA (Sigma, USA), glutamate and malate, DNP, Hepes (Serva, FRG) and TPP^+ (Fluka, Switzerland) were used.

3. RESULTS AND DISCUSSION

The stimulation of respiration and the fall of the membrane potential of liver mitochondria in active ground squirrels, caused by the addition of 20 μM of palmitate, are found to be suppressed by 1 μM CAtr in the presence of oligomycin (Fig. 1a); the same effect was observed in rat liver mitochondria [2,3]. This suggests that the ATP/ADP-antiporter of liver mitochon-

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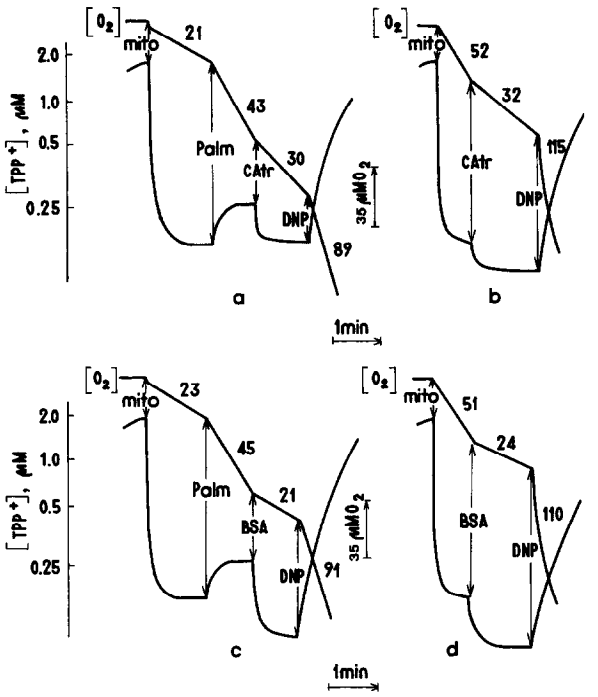

Fig. 1. Effect of palmitate, carboxyatractylate and BSA on the membrane potential and respiration of liver mitochondria from active (aroused) (a,c) and arousing (b,d) ground squirrels. Upper and lower curves, polarograms and [TPP$^+$], respectively. For incubation conditions of mitochondria, see section 2. Incubation mixture was supplemented with 2 μM oligomycin. Additions: 20 μM palmitic acid (Palm), 1 μM carboxyatractylate (CAtr), bovine serum (BSA) albumin (1 mg/ml), 40 μM DNP. Figures above the polarograms indicate the respiration rates, nmol $O_2 \cdot 1\ min^{-1} \cdot 1$ mg protein^{-1}.

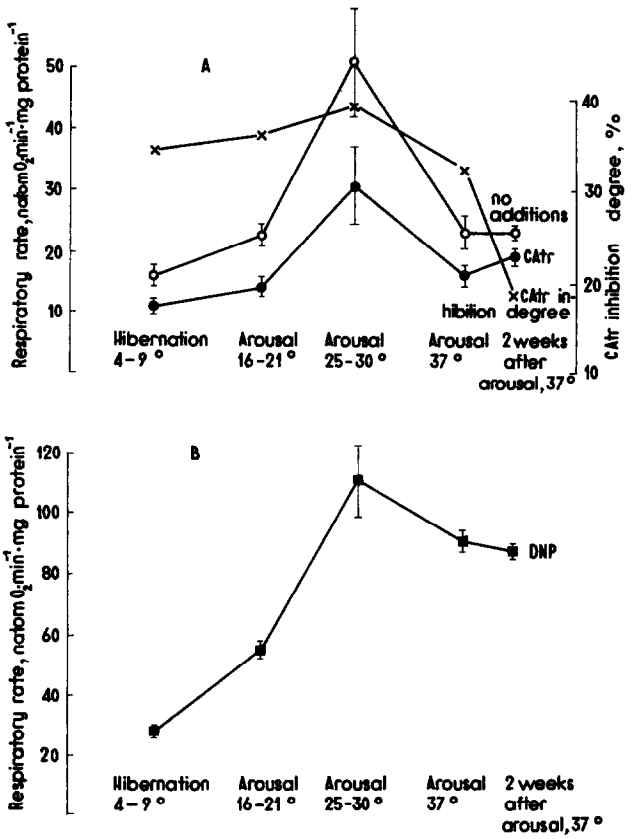

Fig. 2. Energy coupling parameters in liver mitochondria during arousal. The properties of mitochondria from 5 groups of animals (6–7 ground squirrels in each) were compared as follows: hibernating (body temperature, 4–9°C), arousing (16–21°C and 25–30°C), aroused animals (body temperature 37°C, 1 day at 4°C) and a control group, in which the aroused animals were kept for 2 weeks at 20°C. The diagram shows the respiration rates in the presence of 2 μM oligomycin (curve 'No additions'), those in the presence of oligomycin and 1 μM carboxyatractylate (curve 'CAtr'), the degree of respiration suppression by CAtr relative to the respiration rate in the presence of oligomycin without CAtr, and maximal respiratory activities of mitochondria in the presence of 40 μM DNP (curve 'DNP'). Incubation conditions, see in section 2.

dria of ground squirrels participates in the uncoupling by FFAs.

In a similar way, CAtr influences the respiration of mitochondria of arousing ground squirrels (Fig. 1b). The action of BSA appears to be qualitatively analogous with that of CAtr but more pronounced (Figs. 1c,d). The CAtr added after BSA is without any effect (not shown).

Fig. 2 shows several parameters of mitochondria isolated from the liver of ground squirrels at different body temperatures. The mitochondria of the groups compared greatly differ in the maximal respiration rate measured in the presence of DNP (Fig. 2B). This rate is the lowest in mitochondria of the hibernating ground squirrels and reaches the highest values in mitochondria of the arousing animals at body temperature 25–30°C.

The respiration rate in the absence of DNP and in the presence of oligomycin also increases (Fig. 2A). In the presence of oligomycin, CAtr suppresses, to some degree, the respiration of mitochondria. This degree is similar in all groups of animals except for those which were kept after arousel for 2 weeks at 20°C. In the latter case, CAtr suppressed the respiration rate only slightly.

In the same experiments, we also measured the rate of the cyanide-induced discharge of the membrane potential in mitochondria respiring in the presence of oligomycin. This rate, which is known to be proportional to the membrane ion conductance, proved to be about 1.5-fold lower in mitochondria of the hibernating squirrels than in those of the arousing animals. The addition of CAtr lowered the discharge rate in mitochondria of the arousing ground squirrels more than in those of the hibernating ones. A similar effect was observed when BSA substituted for CAtr (not shown).

It should be noted that the lower ion conductance of the inner mitochondrial membrane of hibernating ground squirrels is not at variance with a rather high degree of the suppression of respiration and the membrane potential discharge rates by CAtr in the same mitochondria (see Fig. 2A). The low activity of the respiration chain (Fig. 2B), i.e. the low proton potential generation power, is characteristic of mitochondria of

the hibernating ground squirrels. This explains why even a slight change in the ion conductance of the mitochondrial membrane considerably affects the membrane potential level and the respiration rate.

Maybe a strong decrease in the respiratory chain activity in the mitochondria of hibernating ground squirrels is essential not only for the limited substrate utilization and heat production. The decrease in the respiratory chain activity sharply increases the membrane potential sensitivity to various stimuli changing the conductance of the mitochondrial membrane. This may contribute to stronger sensitivity of oxidative metabolism of the hibernating animals to hormones and neuromediators, in particular, to noradrenaline and glucagon.

Thus, the results obtained demonstrate that during the arousal of ground squirrels, the uncoupling of oxidative phosphorylation and the increase in the respiratory chain activity are parallel processes occurring in liver mitochondria. As it was previously found in our group, the increase in the respiratory activity of mitochondria of the aroused ground squirrels seems to require activation of phospholipase A_2 [13,14]. The same reason might be responsible for the increase in the level of endogenous FFAs in the mitochondrial membrane, which cause the uncoupling of oxidative phosphorylation in aroused ground squirrels. Lipolysis of the neutral fat may be another FFA source. The decrease in the ion conductance by CAtr suggests that the ATP/ADP-antiporter might be involved in the uncoupling of oxidative phosphorylation in liver mitochondria during the arousal of ground squirrels.

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