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Mitochondrial porin regulates the sensitivity of anion carriers to inhibitors

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In mitoplasts, respiratory stimulation by ADP, palmitate, DNP and CCCP and sensitivity of respiration to carboxyatractylate are considerably less pronounced than in mitochondria. Addition of porin-containing preparations (purified outer membranes or solubilized mitochondrial porin) to mitoplasts results in partial restoration of the oxygen consumption and sensitivity to carboxyatractylate (CAT). The uncoupling effect of FCCP in mitoplasts is CAT-resistant and does not depend on added porin. It is suggested that mitochondrial porin may be a natural activator of ADP/ATP antiporter and succinate carrier in mitochondria.

Mitoplast; Outer mitochondrial membrane; Porin; Uncoupler; ATP/ADP antiporter; Succinate carrier

1. INTRODUCTION

Removal of the outer membrane from the mitochondria provokes a reduction in ADP/ATP exchange [1] and respiratory control ratio [2]. Dissociation of contacts between the inner and outer mitochondrial membranes results in a decrease of the state 3 respiration rate while leaving unchanged the state 4 respiration rate [3,4]. Our previous studies have demonstrated that when the outer membrane fraction or porin-containing liposomes are added to mitoplasts the state 3 and DNP-stimulated respiration rates are essentially enhanced [5,6]. Such effects may be due to the changes in the activity of ADP-, substrate-, and/or DNP-translocating systems induced by mitochondrial porin. As found by Skulachev's group the inhibitor of ADP/ATP antiporter, CAT, is also the inhibitor of palmitate- and DNP-stimulated respiration [7,8]. In order to examine the possibility of regulation of mitochondrial transport by the outer membrane and its constituent, porin, the inhibitory analysis of mitochondrial respiration was used. *n*-Butyl malonate, as specific inhibitor of succinate transport [9], and CAT were used in this study.

Analysis of both CAT- and *n*-butyl malonate-titration curves obtained for mitoplasts in the presence and absence of porin-containing preparations enables us to propose that porin can regulate ADP/ATP and succinate transport in mitochondria.

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Abbreviations: DNP, 2,4-dinitrophenol; CCCP, *m*-chlorophenylhydrazonecarbonyl cyanide; FCCP, *p*-trifluoromethoxyphenylhydrazonecarbonyl cyanide; CAT, carboxyatractylate

2. MATERIALS AND METHODS

Rat liver mitochondria were isolated as in [10]. The isolation medium contained 300 mM sucrose, 10 mM Tris-HCl and 1 mM EGTA, pH 7.5. The isolated mitochondria were then washed in the same medium without EGTA. Mitoplasts and crude fractions of outer membranes were obtained from mitochondria subjected to hypoosmitic shock in 10 mM Tris-HCl (0°C, 20 min, pH 7.5) followed by differential centrifugation (10000 × *g*, 20 min and 100000 × *g*, 60 min, respectively).

Then the crude outer membrane was purified by centrifugation in a stepwise sucrose gradient as in [11] and stored at −20°C. The mitoplasts were washed twice in a medium containing 300 mM sucrose and 10 mM Tris-HCl (pH 7.5) and used in

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the polarographic study. Isolation of mitoplasts and outer membranes was carried out under electron microscopic control.

Mitochondrial porin was isolated by solubilization of rat liver mitochondria in 2% (v/v) Triton X-100 followed by chromatography on hydroxyapatite, cellite, and DEAE-cellulose columns as in [12] and stored at −20°C.

Respiration of mitochondria and mitoplasts was measured by polarographic techniques at 25°C. The incubation medium contained 150 mM sucrose, 10 mM Tris-HCl, 50 mM KCl, 3 mM KH_2PO_4, 4 mM succinate, 1 μM rotenone, pH 7.5. The concentration of mitochondrial or mitoplast protein in a polarographic cell (1 ml) was 1.2–2.4 mg/ml.

Tris, EGTA, CCCP, FCCP, palmitate, and oligomycin were from Sigma; ADP was from Reanal; and CAT from Boehringer. Sucrose was recrystallized from ethanol or decationized on Dowex-50 column.

3. RESULTS AND DISCUSSION

As shown in figs 1 and 2, the stimulating effects of 200 μM ADP and 50 μM DNP in mitoplasts are smaller than in mitochondria. The uncoupling by 20 μM palmitate or 5 μM CCCP (but not 2×10^{-7} M FCCP) is also less pronounced in mitoplasts as compared to that reported for mitochondria (fig.3A) [7,8].

The addition of the outer membranes (0.7–1.2 μg/ml) enhances the rates of mitoplast respiration in the presence of ADP, DNP, palmitate, and CCCP (figs 1,2 and 3B), whereas the uncoupling effect of FCCP is independent of the added porin.

Titration of respiration by transport inhibitors (CAT or *n*-butyl malonate) demonstrates a decrease in the sensitivity to the inhibitors after removal of the outer membrane from mitochondria which is reversible upon adding the porin-containing preparations to mitoplasts (figs 1–3). On the other hand, when mitoplast respiration was titrated by malonate, the inhibitor of succinate dehydrogenase, no dependence on the presence of porin was found (not shown). As seen from fig.3, in the case of FCCP-uncoupled respiration, the CAT-induced inhibition is observed neither in the presence nor in the absence of porin.

The effect of 50 μM cytochrome *c* on mitoplast respiration is minor when compared to the effect of 1.2 μg/ml outer membrane (fig.2). Control experiments with 2 μg/ml Triton X-100 (this concentration may be contaminating for solubilized porin) did not reveal any change in the mitoplast respiration (not shown). Thus, it is possible to suggest that the effects of porin-containing prepara-

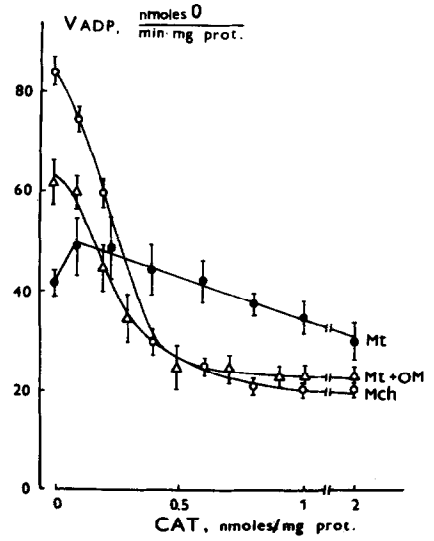

Fig.1. CAT-induced inhibition of state 3 respiration in rat liver mitochondria (Mch), mitoplasts (Mt) and mitoplasts + purified outer membrane (Mt + OM). The incubation medium (see section 2) was supplemented with ADP (200 μM). The concentrations of protein are: Mch, 2.0 mg/ml; Mt, 1.6 mg/ml; OM, 0.7 μg/ml.

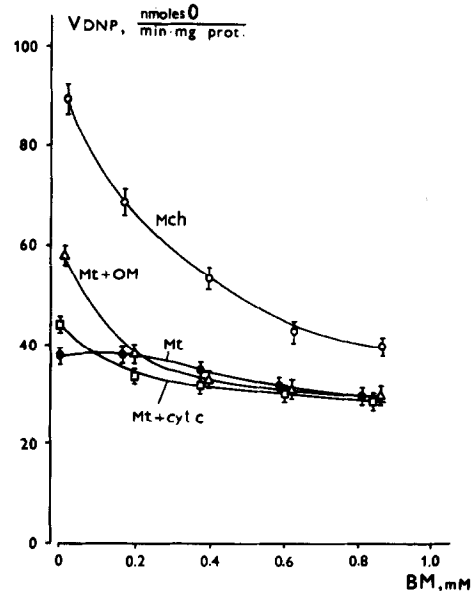

Fig.2. Inhibition of uncoupled respiration by *n*-butyl malonate (BM) in mitochondria (Mch), mitoplasts (Mt), mitoplasts + purified outer membrane (Mt + OM) and mitoplasts + 50 μM cytochrome *c* (Mt + cyt c). The incubation medium (see section 2) was supplemented with 50 μM DNP and 2 μg/ml oligomycin. The concentrations of protein are: Mch, 2.0 mg/ml; Mt, 1.8 mg/ml; OM, 1.5 μg/ml.

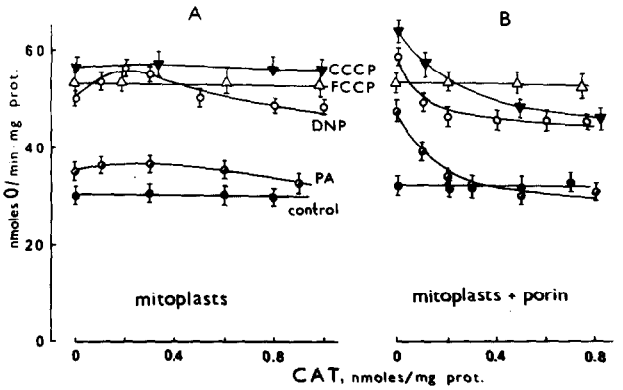

Fig.3. Dependence of uncoupled respiration on CAT concentration in rat liver mitoplasts (2.2 mg protein/ml) in the absence (A) and presence (B) of solubilized mitochondrial porin (0.7 ng/ml). The incubation medium (see section 2) was supplemented with oligomycin (2 μg/ml) and also contained: (▼) 5×10^{-6} M CCCP; (Δ) 2×10^{-7} M FCCP; (○) 5×10^{-6} M DNP; (◐) 2×10^{-5} M palmitate (PA); (●) control.

tions are not due to the presence of the detergent or cytochrome *c*.

According to the data of Krämer [13,14], the ADP/ATP carrier in mitoplasts and reconstituted system (not in mitochondria) can be activated by anion and cation effectors. Thus, the activation of the state 3 and uncoupled respiration in mitoplasts (figs 1 and 3) by low CAT concentrations (0.1–0.5 nmol/mg prot.) may be ascribed to the effect of CAT as a polyvalent anion, whereas positively charged mitochondrial porin [15] can act as a cation activator of the ADP/ATP carrier. As seen from figs 1 and 3, porin enhances the CAT-sensitivity of both ADP-stimulated and uncoupled respiration in mitoplasts, thus demonstrating the porin-induced activation of not only ADP-translocation but also the uncoupling function of ADP/ATP antiporter (the latter function of the antiporter was presented by Skulachev's group [7,8]).

Moreover, the increase in the respiration sensitivity to *n*-butyl malonate (but not to malonate) observed after porin was added to mitoplasts is probably due to the activation of succinate transport by porin.

The data presented in this study provide evidence that mitochondrial porin may serve as a natural activator of ADP/ATP, succinate and probably some other carriers in mitochondria.

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