

The role of adenine nucleotide translocators in regulation of oxidative phosphorylation in heart mitochondria

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The regulative role of adenine nucleotide translocators (ANTs) in oxidative phosphorylation has been estimated by the titration of respiration of isolated rabbit heart mitochondria with carboxyatractyloside in the presence of a non-rate limiting creatine phosphokinase ADP-regenerating system. It has been established that the respiration rate is not controlled by ANTs in the two extreme states, state 3 and state 4. On the other hand, at an intermediate respiration rate (30–70% of the state 3 respiration, which roughly corresponds to that under physiological conditions) the ANT control coefficient had a value of 0.62–0.75. Thus, ANT's seem to play a key role in the regulation of oxidative phosphorylation.

Adenine nucleotide translocator; Oxidative phosphorylation; Mitochondrion; Control coefficient; (Heart)

1. INTRODUCTION

The regulation of oxidative phosphorylation in mitochondria is still a subject of intense discussions. Opinions differ as to whether or not an adenine nucleotide translocator (ANT) is involved in controlling this process [1–5]. The amount of control exerted by an enzyme on pathway flux (J) can be quantitatively characterized by the fractional change in pathway flux, induced by fractional change in concentration or activity (E_i) of the enzyme, provided all other parameters remain constant [6]:

$$c_{E_i}^J = \left. \frac{\delta J/J}{\delta E_i/E_i} \right|_{\text{steady state}}$$

The quantity $c_{E_i}^J$ has been called control coefficient of an i th enzyme on flux J [7]. The sum of control

coefficients of all enzymes or enzyme complexes of the system is equal to unity [6].

To meet the requirement of stationarity, ATP and ADP concentrations should be kept constant [6] while investigating the contribution by various mitochondrial enzymes to the control of oxidative phosphorylation. For this purpose, the incubation medium is supplemented with a system of ADP regeneration, for example glucose and hexokinase [5]. Different stationary rates of mitochondrial respiration between state 3 and state 4 (according to the terminology of Chance and Williams [8]) are adjusted by adding various amounts of hexokinase. The control of respiration under the above-mentioned conditions is shared between mitochondrial enzymes and the ADP-regenerating system, and the control coefficient of ANT's, as well as that of other mitochondrial enzymes, largely depends on the kinetic properties of the ADP-regenerating system [9,10]. This may result in misinterpretation of the experimental data.

Recently it has been established for liver mitochondria that ANT's play an essential role in

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the regulation of oxidative phosphorylation within the physiological region of respiratory rates [11]. However, this has not been studied thoroughly for heart mitochondria. There is an opinion [12,13] that the heart mitochondrial respiration rate is not controlled by ANT's. However, this conclusion is only consistent with the maximal rate of respiration (state 3).

In this paper the evidence concerning the role of ANT's in the control of heart mitochondrial respiration is presented. The creatine phosphokinase system was used for ADP regeneration and experimental conditions were chosen in such a way that the rate of respiration would be regulated only by the mitochondrial enzymes.

2. MATERIALS AND METHODS

Mitochondria from rabbit heart were isolated with trypsin [14]. The respiration rate was measured polarographically with a Clark-type electrode (Radiometer E 504710) at 37°C in a medium, containing 10 mM Tris-HCl, 5 mM KH_2PO_4 , 20 mM succinate, 1 μM rotenone, 10 mM dithiothreitol, 1 mM ATP, 4 IU/ml creatine phosphokinase, 0.5 mM free Mg^{2+} (calculated according to the binding constants [15]), pH 7.2. Different stationary rates of mitochondrial respiration were adjusted by varying the creatine and creatine phosphate ratio in the medium, while the sum of their concentrations was kept constant, 50 mM. The concentration of KCl (see fig.2) was respectively changed for the maintenance of ionic strength. The control coefficient of ANT's was calculated from the data obtained by titration of mitochondrial respiration with carboxyatractyloside [5]. For the determination of extramitochondrial ADP, aliquots of the incubation medium were filtrated through Synpor N 4 membrane filters directly into ice-cold HClO_4 . After neutralization, the ADP concentration was determined using a standard spectrophotometric enzymic assay [16]. Mitochondrial protein was determined by modified biuret method [17].

3. RESULTS AND DISCUSSION

The distinctive feature of these experiments was that the ADP-regenerating system did not control oxidative phosphorylation at all stationary rates of

mitochondrial respiration between state 3 and state 4. The excess of creatine phosphokinase was used so that a further increase in its concentration would not influence the rate of mitochondrial respiration as preset by various initial creatine/creatine phosphate ratios. The total amount of creatine and creatine phosphate (50 mM) was such that ADP concentration would not change more than by 10% throughout the experiment. The measurement of extramitochondrial ADP during carboxyatractyloside titration indicated that this condition was satisfied. Fig.1 shows the recording of oxygen uptake by mitochondria during carboxyatractyloside titration. As can be seen, the second addition of creatine phosphokinase (4 IU/ml) had no influence on the rate of respiration. Thus, the control coefficient of the ADP-regenerating system was equal to zero. Fig.2 demonstrates typical results of a carboxyatractyloside titration at different rates of mitochondrial respiration. The rate of ADP-stimulated respiration (state 3), 442 natom O/min per mg protein, was very similar to that in the presence of 45 mM creatine and 5 mM creatine phosphate. The sigmoidal shape of titration curves indicates that ANT's do not control the oxidation of succinate at the maximal rate of respiration. This is in accordance with the data of other investigators [12,13] and our previous results obtained with the open system. In the case of ADP-stimulated maximal respiration with succinate, the value of the control coefficient for ANT's was also equal to zero [18].

Even the slight inhibition of ANT's led to a significant decrease in the rate of succinate oxida-

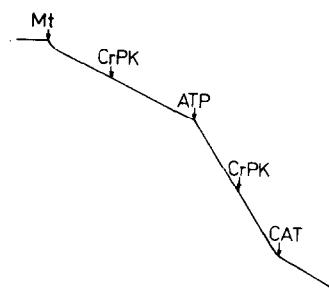


Fig.1. Polarographic recording of mitochondrial respiration during a carboxyatractyloside titration. Mt, mitochondria (0.5 mg/ml); CrPK, creatine phosphokinase; CAT, carboxyatractyloside.

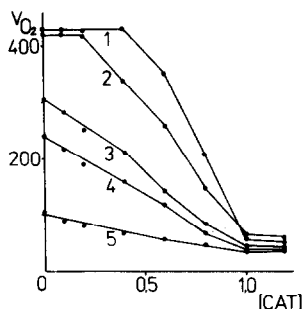


Fig.2. Carboxyatractyloside inhibition of oxygen uptake by mitochondria at different respiration rates. Different steady-state rates of respiration were adjusted by varying creatine and creatine phosphate: (1) 45 mM creatine (Cr), 5 mM creatine phosphate (CrP), 105 mM KCl; (2) 35 mM Cr, 15 mM CrP, 95 mM KCl; (3) 20 mM Cr, 30 mM CrP, 90 mM KCl; (4) 10 mM Cr, 40 mM CrP, 70 mM KCl; (5) 5 mM Cr, 45 mM CrP, 65 mM KCl. V_{O_2} , mitochondrial respiration rate, expressed in natom O/min per mg protein; CAT, carboxyatractyloside, nmol/mg protein.

tion, when the respiration rate constituted 30–70% of maximal. The control coefficient of ANT was estimated by the initial slope of different carboxyatractyloside titration curves. As shown in fig.3, the control coefficient of ANT was high (0.62–0.75) for intermediate states of respiration and approached zero for either state 4 or state 3.

Thus, the main conclusion from the results presented here is that ANT plays an essential role in the regulation of succinate oxidation within the physiological region of heart mitochondrial respiration (30–70% of the maximal rate).

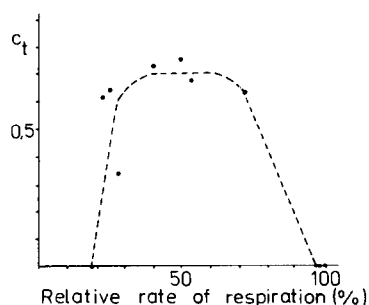


Fig.3. The dependence of the ANT control coefficient on the mitochondrial respiration rate. The V_3 respiration rate is equal to 100%.

Wanders et al. [19], who also used the creatine phosphokinase system for ADP regeneration in experiments on liver mitochondria, have obtained different rates of mitochondrial respiration by varying concentration of creatine phosphokinase against the concentrations of creatine and creatine phosphate kept constant (20 mM and 1 mM, respectively). Consequently, the regenerating system was rate limiting in the region of mitochondrial respiration below 80% of maximal. It is noteworthy that the value of the control coefficient of ANT was close to 0.7, if the control coefficient of creatine phosphokinase was negligible (at the 80% level of the maximal rate).

The experimental system used here simulates the one situation characteristic of energy metabolism in heart cells. The data obtained suggest that ANT is the main step in regulation of oxidative phosphorylation under physiological conditions. In this case, various activators and inhibitors of adenine nucleotide transport (for example, the accumulation of long-chain acyl-CoA during starvation, ischemia and diabetes) will influence the rate of ATP production.

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