ROLE OF AN ADENINE-NUCLEOTIDE TRANSLOCATOR IN REGULATION OF MITOCHONDRIAL PYRUVATE OXIDATION IN THE HEART

L. J. Ivanoviene, V. J. Borutaite,
V. J. Zilinskiene, A. J. Toleikis,
and A. K. Praskevicius

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In recent years the role of a mitochondrial adenine-nucleotide translocator (ANT) in the regulation of oxidative phosphorylation (OP) has been intensively studied. Since adenine nucleotide (AN) transport in mitochondria (MCh) is far from equilibrium [10, 12, 13], this suggests that ANT must control OP, but there is also evidence to the contrary [5, 6]. This contradiction can be explained on the grounds that the controlling ability of ANT is not constant but largely depends on many factors, whose role has not been completely studied. These factors include the rate of mitochondrial respiration [8], the ATP/ADP ratio, and the total mitochondrial AN content [11].

The role of ANT in the regulation of mitochondrial respiration has been studied mainly on isolated MCh or on liver cells. There have been comparatively few studies of this problem on MCh of the heart, and even then, the principal energy-forming substrates of the myocardium were not used [6].

The aim of this investigation was to study regulation of oxidation of pyruvate, one of the principal myocardial substrates, and for comparison, the rate of oxidation of succinate also, under various experimental conditions.

EXPERIMENTAL METHOD

MCh were isolated from the rabbit heart with the aid of trypsin [2]. Respiration of MCh was recorded polarographically with the aid of a covered oxygen electrode in medium containing 0.15 M KCl, 5 mM KH_2PO_4, 10 mM Tris-HCl, and 1.25 mM $MgCl_2$ (pH 7.2). The concentrations of substrates and other reagents were: 1 mM pyruvate + 1 mM malate, 20 mM succinate + 1 μM rotenone, and 600 μM ADP. To exhaust the endogenous substrates MCh were incubated in a polarographic cell for 1.5-2 min in the above-mentioned medium, containing ADP, in the absence of the substrate. Protein was determined by the biuret method [1].

The control strength (C_i) of ANT and of succinate dehydrogenase (SDH) was calculated from titration curves of mitochondrial respiration with carboxyatractylozide (COAO) an irreversible and stoichiometric inhibitor of ANT, and with thenoyltrifluoroacetone (TTFA), an SDH inhibitor, by the equation [8]:

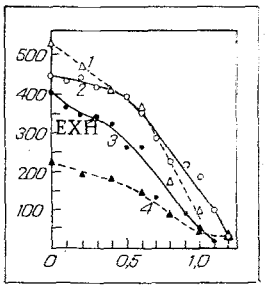

Fig. 1. Dependence of rate of respiration of MCH with pyruvate and malate on COAO concentration under different experimental conditions. 1) "Unexhausted" MCh, ATP/ADP = 5; 2) "unexhausted" MCh; 3) "exhausted" MCh; 4) "unexhausted" MCh, ATP/ADP = 10. EXH) Here and in Figs. 2 and 3: abscissa, COAO concentration (in nmole/mg protein); ordinate, rate of respiration of MCh in state 3 (in nanoatoms O_2/min/mg).

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$$C_i = \frac{dJ/J}{dI/I_{max}},$$

where J is the rate of mitochondrial respiration in the absence of the inhibitor, I_{max} the concentration of inhibitor completely suppressing respiration, and dJ/dI the initial slope of the titration curve.

EXPERIMENTAL RESULTS

The writers showed previously in experiments on cardiac MCh that ANT does not limit oxidation of acetate, palmitoylcarnitine, and succinate [14]. However, it was found that ANT controls respiration of MCh with pyruvate and malate (Fig. 1). It is noteworthy that the control strength of ANT increases from 0.14 to 0.62 during exhaustion of endogenous substrates (parallel experiments). The process of exhaustion of endogenous substrates in MCh ought to lead to an increase in the ATP/ADP ratio in the incubation medium. It will be clear from Fig. 1 that an effect similar to "exhaustion" is obtained with "unexhausted" MCh by an increase in the extramitochondrial ATP/ADP ratio to 5 and 10. For this purpose, before the addition of ADP to the sample, ATP was added in concentrations of 3 and 6 mM respectively. Under these conditions even a comparatively low rate of mitochondrial respiration (ATP/ADP = 10) is controlled by ANT to the same degree as in the case of "exhausted" MCh (C_i = 0.52).

Another picture was obtained when the role of ANT in regulation of succinate oxidation was studied (Fig. 2a). In this case, neither exhaustion of endogenous substrates nor an increase in the extramitochondrial ATP/ADP ratio led to an increase in the control strength of ANT (which remained equal to 0). Experiments with titration of mitochondrial respiration on succinate with TTFA (Fig. 2c) showed that the main stage controlling oxidation of this substrate is SDN (C_i = 0.8). The rate of respiration of MCh with succinate (480-548 nanoatoms O_2/min/mg), incidentally, was similar to and, in some cases, it actually exceeds that with pyruvate and malate. Despite this fact, ANT did not determine the rate of OP with succinate. Our observations thus do not agree with those of Tager et al. [11], who found that the control force of ANT is directly dependent on the rate of respiration in experiments on rat liver MCh. To study this problem in more detail, we carried out experiments to titrate mitochondrial respiration on pyruvate and malate with COAO, when depressed by about 50% by rotenone, a respiratory chain inhibitor, or by oligomycin, an ATPase inhibitor (Fig. 3). When ATPase activity was reduced, the controlling capacity of ANT was found to be unchanged (C_i = 0.57 ± 0.08). Partial inhibition of the respiratory chain by rotenone reduced the control strength of ANT by 22% (C_i = 0.46 ± 0.03; $p < 0.02$). Consequently, the control strength of ANT depends on the method of limiting the respiration rate of MCh, in agreement with the results of an investigation [4] conducted on rat heart MCh.

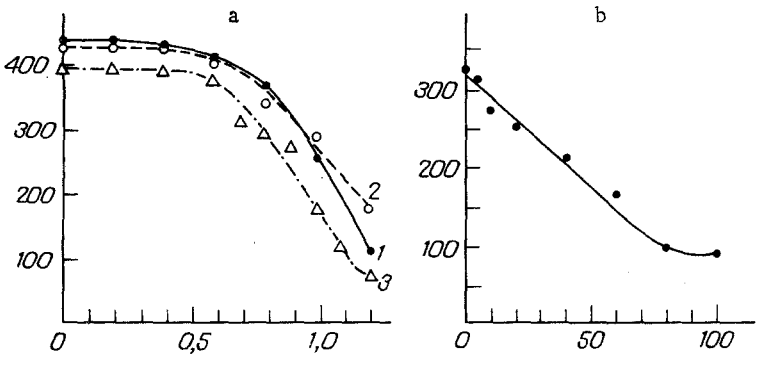

Fig. 2

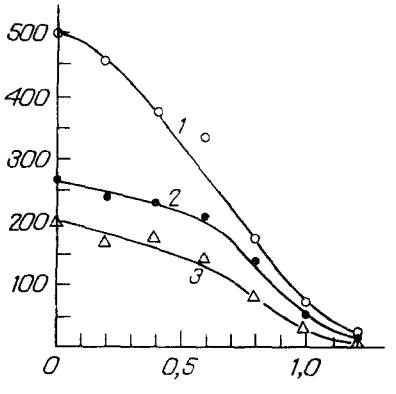

Fig. 3

Fig. 2. Dependence of respiration rate of MCh with succinate on COAO (a) and TTFA (b) concentration. 1) "Unexhausted" MCh, ATP/ADP = 1; 2) "unexhausted" MCh, ATP/ADP = 5; 3) "exhausted" MCh. TTFA concentration given in nmoles/mg protein.

Fig. 3. Dependence of respiration rate of MCh with pyruvate and malate on COAO concentration in the presence of rotenone and oligomycin. 1) Control; 2) respiration inhibited by rotenone; 3) respiration inhibited by oligomycin. Respiration of MCh recorded after exhaustion of endogenous substrates.

Taken as a whole the data show that the respiration rate of MCh is not the chief factor determining the regulatory function of ANT. The ATP/ADP ratio, regarded as a possible metabolic signal determining changes in OP activity [10], may be more important. The respiration rate of MCh has been shown to depend on the extramitochondrial ATP/ADP ratio within the range 5:100 [10]. According to a mathematical model of OP, mitochondrial respiration ought to be more sensitive to the intramitochondrial ATP/ADP ratio [3]. However, because of technical difficulties in the precise determination of real concentrations of intramitochondrial AN, this hypothesis has not yet been proved experimentally.

The distribution of control strengths between individual components of the metabolic pathway is determined by the complexity of the polyenzyme system [7]. The succinate oxidation system is less complex than the pyruvate oxidation system, and the main role in respiration control is played by SDH (C_i = 0.82). During oxidation of pyruvate, control is distributed differently, i.e., ANT largely controls OP, but the control strength of the translocator, in absolute terms (C_i = 0.62), is lower than that for SDH in the succinate-oxidase system. According to Kacser and Burns [9], the sum of the control strengths of all the enzymes of the system is 1. It seems likely that besides ANT, an important role in the regulation of pyruvate oxidation may also be played by other components: ATPase, the respiratory chain, the Krebs cycle, etc.

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