Quantification of the role of the adenine nucleotide translocator in the control of mitochondrial respiration in isolated rat-liver cells

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Received 28 June 1982

Carboxyatractyloside *Adenine nucleotide translocation* *Control strength* *Hepatocyte* *Hepatocyte respiration* *Mitochondrial respiration*

1. INTRODUCTION

In liver cells, the ATP formed during mitochondrial oxidative phosphorylation is utilized either for ATP-requiring reactions in the mitochondrial matrix or, after transport to the cytosol via the adenine nucleotide translocator, for endergonic reactions in the extramitochondrial compartments. Although it is clear that the rate of mitochondrial respiration varies in relation to the ATP requirements of the cell, the way in which control of respiration is achieved is still a matter of controversy.

Particular attention has been focussed on the role of the cytochrome oxidase step and the adenine nucleotide translocator in controlling respiration. Wilson and coworkers (review [1]) have concluded that the first two phosphorylation sites of the respiratory chain are in near-equilibrium with the extramitochondrial phosphate potential and that regulation of respiration occurs at the cytochrome oxidase step, the effective rate constant of this reaction being dependent on the extramitochondrial phosphate potential. According to this model, the adenine nucleotide translocator does not exert significant control on respiration. However, Akerboom and coworkers [2,3] have concluded from the results of inhibition studies with atractyloside that the adenine nucleotide translocator is a rate-controlling step for oxidative phosphorylation in rat-liver cells. In contrast, Stubbs et al. [4], who have obtained similar results to those in [2], have come to the opposite conclusion (see also [5–7]). This apparent contradiction illustrates the ambiguity of conclusions about rate control based solely on the shape of an inhibition curve.

We have shown [8] that, by applying the control theory developed in [9,10], inhibitors can be used in an unequivocal way to quantify the amount of control which a step in a metabolic pathway exerts on flux through that pathway. Using this approach, we have quantified the contribution of different steps to the control of respiration in isolated rat-liver mitochondria [11]. The results of the study showed that the amount of control (control strength) of the adenine nucleotide translocator depends on the rate of respiration on the one hand, and on the ADP-regenerating system used on the other. We have now employed the same approach to measure the control strength of the adenine nucleotide translocator in isolated rat-liver cells. In addition we have examined the controversial question (review [12]) of whether long-chain fatty acyl-CoA esters inhibit the adenine nucleotide translocator in the intact cell in a manner analogous to that demonstrated for isolated rat-liver mitochondria [13–19]. The results are described here.

2. METHODS AND MATERIALS

Parenchymal cells were isolated from the livers of 24-h-fasted male Wistar rats by the method of Berry and Friend [20] as modified [21]. The cells were incubated in Krebs–Henseleit bicarbonate

Abbreviation: CAT, carboxyatractyloside

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Published by Elsevier Biomedical Press

00145793/82/0000–0000/$2.75

buffer containing 2.5 mM Ca^{2+} and supplemented with 2% (w/v) dialysed bovine serum albumin. The incubations were done at 37°C in 25-ml plastic Erlenmeyer flasks with 95% O_2:5% CO_2 as the gas phase.

For measurement of the rate of O_2 uptake, 1.8 ml cell suspension was transferred from the Erlenmeyer flask to a 1.8 ml vessel equipped with a Clark electrode and thermostatically controlled at 37°C.

Collagenase (type IV), carboxyatractyloside (CAT) and antimycin A were obtained from Sigma Chemical Co. (St Louis, MO). All other reagents were of analytical grade.

3. RESULTS

In [11], the control strength of the adenine nucleotide translocator on respiration in isolated rat-liver mitochondria was determined by using carboxyatractyloside (CAT), a specific inhibitor of the translocator. Since CAT is an irreversible inhibitor, the control strength can be calculated directly from the inhibition curve using the relationship (see [8]):

$$C = (dJ/J) / (dI/I_{max}) \quad (1)$$

where:

J = the flux through the pathway at zero inhibitor concentration;
I = the concentration of inhibitor;
I_{max} = the concentration of inhibitor required for full inhibition of flux through the translocator;
dJ/dI = the initial slope of the inhibition curve.

A complication in using CAT in intact cells is that the inhibitor does not permeate rapidly through the plasma membrane (see [4,5]). However, if transport of CAT is brought about by passive diffusion so that there is a linear relationship between the extracellular concentration of the inhibitor and its rate of permeation, it should be possible to use the inhibitor to measure the control strength of the adenine nucleotide translocator in the intact cell. The following experiment was done to test this possibility.

Rat-liver cells were incubated with CAT for 20 min or 40 min, after which the rate of respiration was measured. Fig.1A shows that the inhibition of respiration by CAT was more effective after

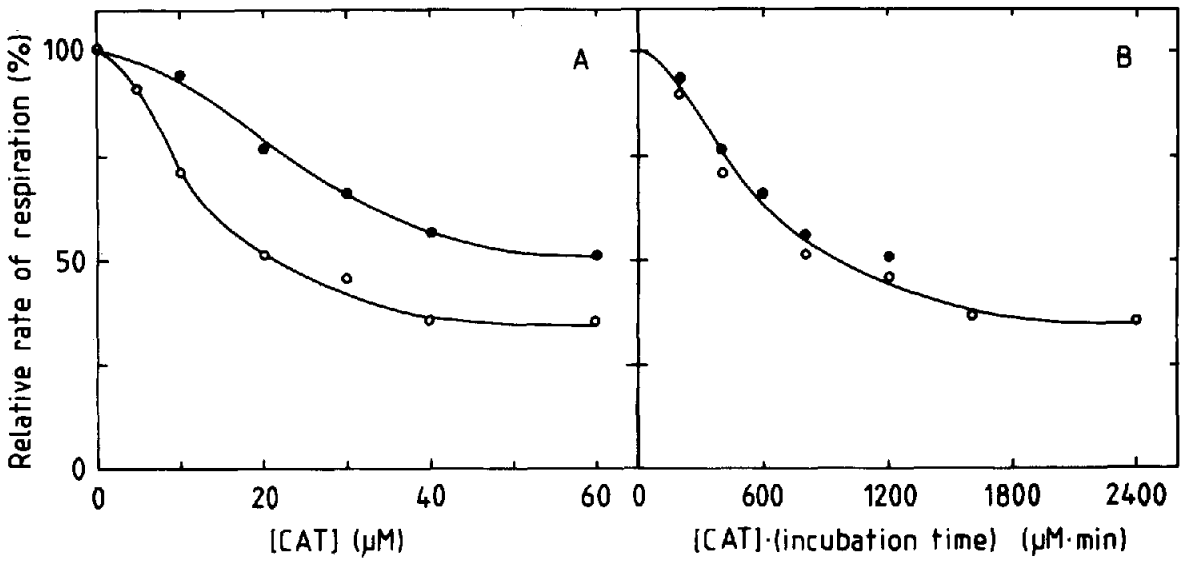

Fig.1. Relationship between the rate of oxygen uptake in rat-liver cells and (A) the concentration of carboxyatractyloside after incubation for 20 or 40 min or (B) the product of carboxyatractyloside concentration and incubation time. Isolated rat-liver cells (10 mg/ml) were incubated with 10 mM lactate plus 1 mM pyruvate and different concentrations of carboxyatractyloside for 20 (•—•) or 40 (○—○) min. At the end of the incubation time the rate of oxygen uptake was measured as in section 2.

40 min than after 20 min incubation, in agreement with the slow permeation of the inhibitor through the plasma membrane. When the rate of respiration is plotted as a function of the product of the inhibitor concentration and the incubation time, the points fall on a single curve (fig.1B), suggesting that the transport of CAT across the plasma membrane is brought about solely by diffusion.

Thus, under the conditions of the experiment of fig.1, the following relationship holds:

$$CAT_{in} = k \cdot t \cdot [CAT]_{out} \quad (2)$$

where:

CAT_{in} = the amount of CAT in the cells (bound to the translocator);

$[CAT]_{out}$ = the extracellular concentration of CAT;

t = the time of incubation;

k = a constant.

In view of the relationship shown in eq. (2), the following expression for the control strength of the adenine nucleotide translocator in intact liver cells can be derived from eq. (1):

$$C = \frac{dJ/J}{d[CAT]_{out}/([CAT]_{out})_{max}} \quad (3)$$

where:

$[CAT]_{out}$ = the extracellular concentration of CAT;

$([CAT]_{out})_{max}$ = the extracellular concentration of CAT which completely inhibits adenine nucleotide translocator-related O_2 uptake under the conditions used;

J = the rate of mitochondrial respiration.

Fig.2 shows the results of a typical experiment in which the control strength of the adenine nucleotide translocator was determined. The cells were incubated for 30 min with lactate *plus* pyruvate and different concentrations of CAT. The minimum amount of CAT required for complete inhibition of adenine nucleotide translocator-related respiration was obtained by extrapolation as indicated in fig.2 (see also [8]). Since non-mitochondrial processes contribute to total O_2 consumption in liver cells, the mitochondrial contribution was determined by using antimycin, a specific

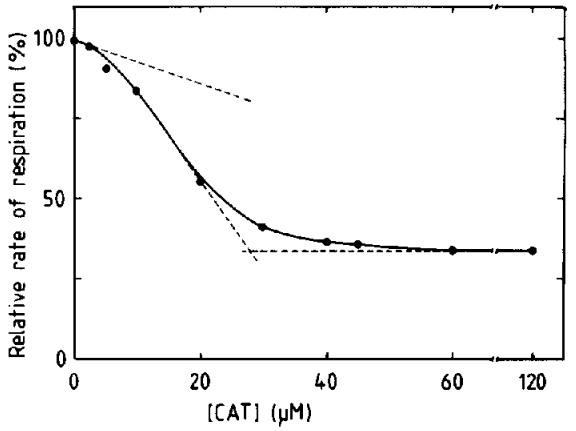

Fig.2. Determination of the control strength of the adenine nucleotide translocator on mitochondrial respiration in isolated rat-liver cells. The cells (10 mg/ml) were incubated with 10 mM lactate plus 1 mM pyruvate and different concentrations of carboxyatractyloside. After 30 min the rate of oxygen uptake was measured as in section 2. The control strength of the adenine nucleotide translocator on respiration was calculated from eq. (3). The initial slope of the curve ($dJ/d[CAT]_{out}$) and the value for $([CAT]_{out})_{max}$ were derived as shown in the figure. The value for J (mitochondrial respiration) was corrected for non-mitochondrial respiration. Non-mitochondrial respiration was determined by addition of an excess of antimycin (70 μM) to the oxygraph vessel and, in the absence of CAT, accounted for 20% ± 1% (mean ± SD, n = 6) of the rate of oxygen uptake. Results of a typical experiment are shown. Analogous results were obtained in 5 other experiments.

inhibitor of the mitochondrial respiratory chain (see [22]). It should be noted that the rate of O_2 uptake in the presence of antimycin is less than that in the presence of an excess of CAT (see fig.2); the difference represents state 4 respiration in the nomenclature of [23].

Using eq. (3) and the data of experiments like that of fig.2 carried out with 6 different cell preparations, a value of 0.26 ± 0.01 (mean ± SEM) is obtained for the control strength of the adenine nucleotide translocator in isolated rat-liver cells under these conditions.

It has been suggested that long-chain acyl-CoA esters may inhibit the translocator in rat-liver cells [24]. To test this, experiments like those in fig.2 were carried out in the presence of oleate, which is known to increase the content of long-chain acyl-

CoA in rat-liver cells (see, e.g., [25]). The control strength of the adenine nucleotide translocator in the presence of oleate (0.24 ± 0.02 (mean ± SEM; $n = 5$)) was approximately the same as that in the absence of oleate.

4. DISCUSSION

In [7] Stubbs has concluded that it is unlikely that the adenine nucleotide translocator is rate-limiting under physiological conditions. While we agree that the adenine nucleotide translocator is not rate-limiting (i.e., 100% control by this step), the quantification described above indicates that the contribution of the translocator to flux control is ~25%.

One of the arguments brought forward in [7] in favour of the thesis that the adenine nucleotide translocator does not exert significant control on respiration is that the first 2 sites of the respiratory chain are in near-equilibrium with the cytosolic phosphate potential, so that the adenine nucleotide translocator must also be in near equilibrium. However, the question of whether the adenine nucleotide translocator is in near-equilibrium, as suggested in [1], or displaced from equilibrium, as shown by direct measurements in actively respiring isolated mitochondria [26–28], need not be directly related to the amount of control exerted by this step. Indeed, Kacser and Burns [9] have shown that even a near-equilibrium step can exert significant control on flux through a pathway, depending on the nature of the preceding and subsequent steps.

The quantitative approach described here has enabled us to examine the question of whether long-chain fatty acyl-CoA esters inhibit adenine nucleotide transport in the intact cell. The following observations indicate that this is not the case:

(1) The amount of carboxyatractyloside required for maximal inhibition of the translocator is the same in the presence as in the absence of oleate (not shown).

(2) The control strength of the translocator was not changed by the addition of oleate. The apparent difference between isolated mitochondria and intact cells with regard to the effect of long-chain acyl-CoA esters on adenine nucleotide transport is presumably due to binding of the esters to components in the cytosol.

ACKNOWLEDGEMENTS

The authors are grateful to Karel Auwerda for helpful discussions. This study was supported by a grant from the Netherlands Organization for the Advancement of Pure Research (ZWO) under the auspices of the Netherlands Foundation for Chemical Research (SON).

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