Biochimica et Biophysica Acta, 333 (1974) 577—580
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BBA Report

BBA 41253

OXIDATION OF REDUCED NICOTINAMIDE-ADENINE DINUCLEOTIDE BY THE MALATE—ASPARTATE SHUTTLE IN EHRLICH ASCITES TUMOUR CELLS

O. DIONISI, G. LONGHI, M.L. EBOLI, T. GALEOTTI and T. TERRANOVA

Institute of General Pathology, Catholic University, Rome (Italy)*

(Received December 27th, 1973)

Summary

The capability of ascites tumour mitochondria to oxidize externally formed NADH has been investigated in intact cells. Lactate has been used as the source of reducing equivalents and the oxidation of this substrate to pyruvate has been estimated. Ascites cells, under conditions of endogenous metabolism, are able to produce pyruvate upon addition of lactate. This effect is prevented by aminooxyacetate, an inhibitor of glutamate—oxalacetate transaminase (EC 2.6.1.1). Half-maximal inhibition by aminooxyacetate is attained at a concentration of approx. 30 μM. Oxidation of lactate is also sensitive to inhibitors of mitochondrial electron and energy transfer and it is enhanced by α-oxoglutarate plus aspartate. These data demonstrate that reducing equivalents can be transported across the mitochondrial membrane of intact Ehrlich ascites tumour cells by the malate—aspartate shuttle.

It has been suggested that the high rate of aerobic glycolysis in tumour cells depends on the lack of systems (substrate shuttles) for the mitochondrial oxidation of cytosolic reducing equivalents [1—3]. Previous work in our laboratory has provided evidence that in Ehrlich ascites tumour cells this is not the case, at least as far as their ability to re-oxidize glycolytic NADH in the mitochondria beyond the first phosphorylation site is concerned [4]. Further studies along this line have also shown the possibility that the malate—aspartate shuttle [5] plays a role in bringing about transport of reducing equivalents across the mitochondrial membrane of ascites cells [6—9]. How-

Abbreviation: ELD cells, Ehrlich—Lettré hyperdiploid ascites tumour cells.

*Postal address: via Pineta Sacchetti 644, 00168 Roma, Italy.

ever, no direct evidence was derived from these studies that such a shuttle is actually operating in intact cells.

An experimental system, using lactate as substrate, similar to that already tested in Landschuetz ascites tumour cells [10, 11], was thought to be suitable for evaluating the functional significance of the malate—aspartate shuttle in Ehrlich ascites cells. The results obtained show that the oxidation of lactate to pyruvate does involve the malate—aspartate shuttle. Thus, we feel that other factors, unrelated to the mechanism of transport of cytosolic reducing power to the respiratory chain, are responsible for the high rate of NADH oxidation at the lactate dehydrogenase (EC 1.1.1.27) level in glycolyzing Ehrlich ascites cells.

Ehrlich hyperdiploid ascites tumour cells of the Lettré strain were grown and prepared as described earlier [12]. Pyruvate was analysed enzymically in neutralized $HClO_4$ extracts [13]. O_2 consumption was measured manometrically at 38 °C by the conventional Warburg method, using 0.2 ml KOH in the centre well and 3 ml of cell suspension (5—10 mg dry wt/ml) in the main compartment of the manometric flasks. Further experimental details are given in the legend to Fig. 1. Aminooxyacetic acid hydrochloride and rotenone were obtained by K & K Lab. Inc. Aminooxyacetate was neutralised to pH 7.4 before use. The 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (TTFB) was kindly supplied by Dr R.B. Beechey of Shell Research Ltd. NADH and lactate dehydrogenase were purchased from Boehringer und Soehne. All other chemicals were products of Sigma Chemical Co. or E. Merck.

Fig. 1 shows the kinetics of the oxidation of lactate to pyruvate in intact ELD cells. The effects of aminooxyacetate and substrates of the malate—aspartate shuttle on the lactate oxidation are also illustrated in this Figure. Addition of excess lactate results in the production of about 10 μmoles pyruvate/g dry wt in 15 min (Fig. 1A) and this is increased by 35% in the presence of α-oxoglutarate plus aspartate (Fig. 1B). Pretreatment of the cells with 0.2 mM aminooxyacetate markedly inhibits pyruvate production both in the absence and the presence of the two substrates. A titration curve of aminooxyacetate inhibition is presented in Fig. 2. The inhibitory effect is almost complete with 0.1 mM aminooxyacetate and half-maximal inhibition is roughly calculated to be at a concentration of 30 μM. It should be noted that no effect on the endogenous respiration of ELD cells was found with concentrations of aminooxyacetate up to 0.4 mM (not shown).

The above results clearly indicate that the oxidation of lactate to pyruvate is linked to the regeneration of NAD via an active malate—aspartate shuttle. This is further documented by the data of Table I which show that the production of pyruvate is prevented by inhibitors of mitochondrial electron and energy transfer. The effect of the uncoupler tetrachlorotrifluoromethylbenzimidazole is probably due to the inhibition of aspartate efflux from the mitochondria, a process which has been shown in rat-heart mitochondria, transaminating glutamate, to require both energy and extramitochondrial phosphate [15, 16].

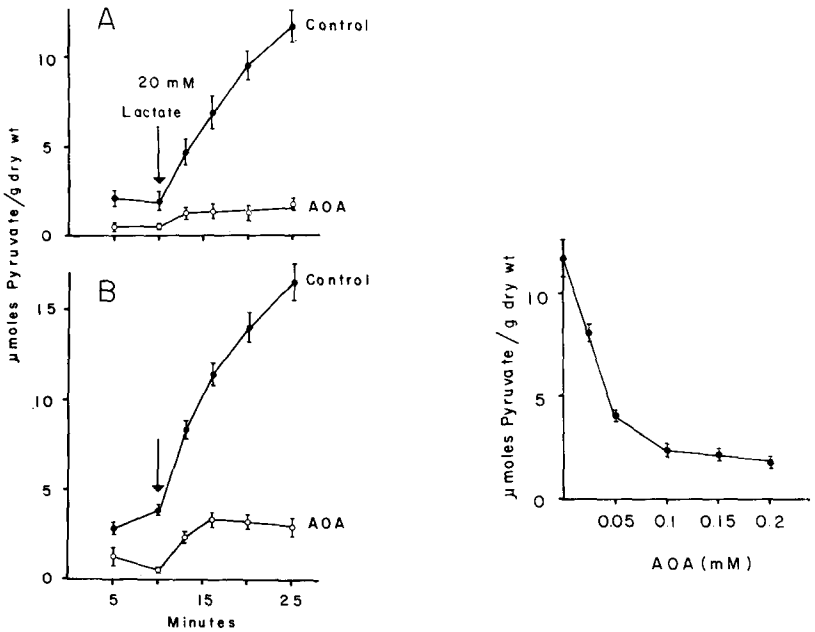

Fig. 1. Pyruvate production upon addition of lactate to Ehrlich—Lettré ascites cells. Erlenmeyer flasks containing 15 ml of cell suspension (20—30 mg dry wt/ml) were incubated at 30 °C under air tension in a Dubnoff shaker. The cells were suspended in an isotonic saline medium of the following composition: 154 mM NaCl, 6.2 mM KCl and 11 mM sodium phosphate buffer, pH 7.4 [14]. A, untreated cells; B, cells treated with α-oxoglutarate plus aspartate. Aminooxyacetate (AOA, 0.2 mM), α-oxoglutarate (10 mM) and aspartate (10 mM) were added at time = 0. The lactate (DL-lactate) addition is indicated by the arrow. 1 ml of cell suspension was withdrawn at intervals and poured in a centrifuge tube containing the same volume of 6% (w/v) ice-cold $HClO_4$. The deproteinized material was then centrifuged and the supernatant neutralized with 0.5 M triethanolamine—3M K_2CO_3 mixture. Pyruvate was assayed enzymically in the neutralized extracts [13]. The vertical lines correspond to twice the standard error of the mean of 5—17 experiments.

Fig. 2. The effect of different concentrations of aminooxyacetate (AOA) on pyruvate production after 15 min incubation of Ehrlich—Lettré ascites cells with 20 mM DL-lactate. The experimental conditions were those described in Fig. 1. The cell suspension concentrations were 20—30 mg dry wt/ml. Each point is the mean ± S.E. of 4—10 experiments.

TABLE I

THE EFFECT OF INHIBITORS OF MITOCHONDRIAL ELECTRON AND ENERGY TRANSFER ON THE LACTATE OXIDATION BY EHRLICH—LETTRÉ ASCITES TUMOUR CELLS

Ascites cells (7—18 mg dry wt/ml) were incubated at 30 °C with 20 mM DL-lactate and different inhibitors. After 15 min incubation, samples were taken and analysed for pyruvate. For other conditions see Fig. 1. Results are expressed as mean ± S.E. (number of observations).

Inhibitors	Pyruvate production (µmoles/g dry wt per 15 min)
None	11.7 ± 0.32 (8)
Rotenone (10 µM)	2.0 ± 0.11 (8)
Antimycin A (15 µg/ml)	0.6 ± 0.05 (8)
Nitrogen	0.4 ± 0.03 (8)
Oligomycin (10 µg/ml)	3.1 ± 0.42 (7)
4,5,6,7-Tetrachloro-2-trifluoromethylbenzimidazole (10 µM)	1.5 ± 0.11 (9)

In conclusion, it can be stated that Ehrlich—Lettré ascites tumour cells, under conditions of endogenous metabolism, are able to carry out an intramitochondrial oxidation of cytosol NADH. The malate—aspartate shuttle is involved to a great extent in this mechanism. Whether the situation is the same in actively glycolyzing cells, where pyruvate is present in large amounts and may compete with the hydrogen shuttles for the reoxidation of NADH, remains to be established.

The skilful technical assistance of Mr Giuseppe Festuccia is gratefully acknowledged. The work was in part supported by a grant from Consiglio Nazionale delle Ricerche, Italy.

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