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THE OPERATION OF THE MALATE-ASPARTATE SHUTTLE IN THE REOXIDATION OF GLYCOLYTIC NADH IN SLICES OF FETAL RAT LIVER

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Summary

Lactate production by liver slices from fetal rats (17th—18th day of gestation) is enhanced about two fold by aminooxyacetate, an inhibitor of aspartate transaminase (EC 2.6.1.1). Such an effect is consistent with an increase of the cytosolic NAD-redox state owing to the parallel fall in the pyruvate level, whereas the glycolytic flux does not seem to be influenced appreciably. Indeed, although the inhibitor causes a marked increase of fructose 1,6-diphosphate, glucose 6-phosphate decreases only slightly. These results suggest that in fetal rat liver the malate-aspartate shuttle is operative in the reoxidation of cytosolic NADH produced during aerobic glycolysis.

Several indications have been obtained up to now that shuttle mechanisms are operating in intact cells of tissues such as heart [1, 2], liver [3—7] and in Ehrlich ascites tumour cells [8—11]. Experiments performed with perfused liver and isolated liver cells from adult rats indicate that channelling of cytosolic reducing equivalents to the mitochondria is rate limiting in the metabolism of glycerol, sorbitol [6], xylitol [4] and ethanol [3,5,7]. In such studies the use of transaminase inhibitors such as aminooxyacetate, DL-cycloserine and difluorooxaloacetate, has been considered a useful tool in estimating the contribution of the malate-aspartate shuttle [12] with respect to other hydrogen-translocating systems.

Although considerable information is available on adult rat liver, similar data have not yet been obtained in fetal rat liver. This tissue, due to its high glycolytic capacity [13], generates an excess of NADH at the level of glyceraldehyde-phosphate dehydrogenase (EC 1. 2.1.12). Thereby one might speculate that the larger amount of lactate accumulated under aerobic condi-

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tions with respect to adult liver is due to inadequacy of the mechanisms responsible for hydrogen translocation to the mitochondria. A similar suggestion has been advanced by Boxer and Devlin [14] for tumour cells but it has been proved by us not to be true at least for highly glycolysing strains of Ehrlich ascites cells [8,10].

In the present work, evidence is presented in favour of the occurrence of the malate-aspartate shuttle in liver slices from fetal rats. Thus, also for these cells the view is challenged that a defect in the removal of reducing power from the cytosol to the mitochondria is responsible for the high rate of lactate production during aerobic glycolysis.

Slices, 0.2—0.5 mm thick, were prepared freehand with a razor blade guided by a glass slide from the liver of late fetal (17th—18th day of gestation) rats of the Wistar strain. Slices (15—30 mg dry weight) were incubated 70 min at 38°C in the main compartments of Warburg flasks containing 3 ml of O_2-saturated medium. The composition of the medium was the following: 140 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 10 mM sodium phosphate, pH 7.4 [15]. 20 mM glucose was present in all the experiments. O_2 consumption was measured manometrically by the conventional Warburg method using 0.2 ml of 20% KOH in the centre well. After completion of the incubation period the medium was assayed for lactate and the slices for ATP, glycolytic intermediates and protein. 1 ml of the medium was added to the same volume of 6% (w/v) ice-cold $HClO_4$. The slices were deproteinized in 8% w/v $HClO_4$ (at 1°C), homogenized and centrifuged. The pellets were assayed for total protein by the biuret reaction [16]. ATP and metabolites were analysed enzymically in the $HClO_4$ extracts neutralised with a mixture of 0.5 M triethanolamine/3 M K_2CO_3 [17]. Aminooxyacetic acid hydrochloride was obtained by K&K Lab. Inc. and was neutralised to pH 7.4 before use. Enzymes, coenzymes and substrates for metabolite determination were obtained from Boehringer und Soehne. All other chemicals were products of E. Merck.

Liver slices from fetal rats produce about
80 nmol lactate/mg dry weight per hour during aerobic glycolysis. As is shown in Fig. 1, this value is increased more than 2 fold by aminooxyacetate. The stimulation is complete at 0.15 mM aminooxyacetate and the half-maximal effect is calculated to be at a concentration of about 25 μM. This result indicates that the increased lactate accumulation may be caused by an increased availability of reduced nicotinamide-adenine dinucleotide in the cytosol as a con-

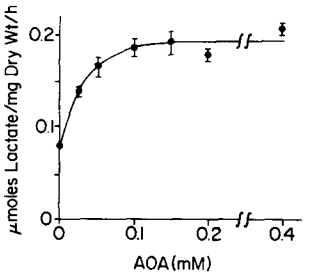

Fig. 1. The effect of different concentrations of aminooxyacetate (AOA) on lactate accumulation in liver slices from 17—18-day-old fetal rats. The points represent mean ± standard error of mean (3—8 experiments).

TABLE I

EFFECTS OF AMINOOXYACETATE ON THE LEVELS OF ATP AND GLYCOLYTIC INTERMEDIATES AND ON THE CYTOSOLIC NAD-REDOX STATE IN LIVER SLICES FROM FETAL RATS

Slices from 17—18-day-old fetal rats were analysed for their content in ATP, glucose 6-phosphate (Glc-6-*P*), fructose 1,6-diphosphate (Fru-1,6-P_2), pyruvate and lactate after 70 min aerobic incubation at 38°C in the absence and presence of aminooxyacetate. The cytosolic NADH/NAD ratio was calculated from the lactate/pyruvate ratio, using an equilibrium constant for lactate dehydrogenase of $1.1 \cdot 10^{-4}$ [18] For other experimental details see the text. The values represent the means ± S.E. (number of observations).

Conditions	nmol · mg^{-1} protein					NADH/NAD (× 10^{-3})
	ATP	Glc-6-*P*	Fru-1,6-P_2	Pyruvate	Lactate	
Control	24.6±2.7(6)	8.7±0.75(6)	1.8±0.4(6)	3.8(2)	165.9±4.2(8)	4.8
Aminooxyacetate (0.4 mM)*	21.2±1.8(7)	6.5±0.85(7)	5.7±0.5(7)	0.9(2)	432.6±10.5(3)	52.9

*Significantly different from values without aminooxyacetate, by Student's *t* test, $P < 0.0125$ (ATP), $P < 0.0005$ (Glc-6-*P*), $P < 0.0005$ (Fru-1,6-P_2), $P < 0.0025$ (pyruvate) and $P < 0.0005$ (lactate).

sequence of the inhibition of hydrogen transfer into mitochondria via the malate-aspartate shuttle. Measurements of ATP and some glycolytic intermediates were also performed in fetal liver slices. As can be seen in Table I, aminooxyacetate influences significantly the level of fructose 1,6-diphosphate (Fru-1,6-P_2) and pyruvate, whereas the content of ATP and glucose 6-phosphate (Glc-6-*P*) diminishes moderately. Similar results have been obtained by Safer et al. [1] in perfused rat heart with glucose as substrate after addition of aminooxyacetate. The small change in the ATP level is likely due to the slight inhibition (about 20%) of O_2 uptake induced by the inhibitor (not shown). The lowering of pyruvate associated with the stimulation in the lactate accumulation is indicative of an increase of the cytosolic redox potential. Indeed the NADH/NAD ratio is enhanced by about 11 times, going from a value of 4.8 to a value of $52.9 \cdot 10^{-3}$. On the other hand, the slight decrease of Glc-6-*P*, despite the marked increase of Fru-1,6-P_2, together with the failure of a rise in the ATP synthesis, suggests that net flux through the glycolytic pathway is not much influenced by aminooxyacetate. Thus we interpret the elevation in the Fru-1,6-P_2 level as due to the inhibition of glyceraldehyde-phosphate dehydrogenase produced by the elevated NADH/NAD ratio in the cytosol.

In conclusion, the present results suggest that in fetal rat liver the excess of cytosolic NADH produced during aerobic glycolysis is reoxidized by the mitochondria through the operation of the malate-aspartate shuttle. This situation resembles that of adult liver where, although aerobic glycolysis is lower, the removal of the excess of cytosolic reducing equivalents by the hydrogen-translocating shuttles is the rate-limiting step in the metabolism of substrates like glycerol, sorbitol, xylitol or ethanol.

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