THE INVOLVEMENT OF MITOCHONDRIAL PYRUVATE TRANSPORT IN THE PATHWAYS OF GLUCONEOGENESIS FROM SERINE AND ALANINE IN ISOLATED RAT AND MOUSE LIVER CELLS

J. MENDES-MOURÃO*, A. P. HALESTRAP, D. M. CRISP** and C. I. POGSON***
Department of Biochemistry, Medical School, University of Bristol, Bristol, BS8 1TD, UK

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1. Introduction

Serine and alanine are both substrates for gluconeogenesis in the rat liver [1–3]. The commonly accepted pathways of gluconeogenesis from both these substrates involves the formation of cytoplasmic pyruvate, in the case of serine by the action of serine dehydratase (EC 4.2.1.13) and in the case of alanine by the action of glutamate pyruvate transaminase (EC 2.6.1.2.) [4]. However, some workers have suggested that serine may be transaminated to hydroxypyruvate thus entering the conventional gluconeogenic pathway at 2-phosphoglycerate [5,6]. Their conclusions were based on the use of quinolinate, an inhibitor of phosphoenolpyruvate carboxykinase [7], but have not been supported by the work of others [1,8]. Alanine is normally thought to be transaminated to pyruvate in the cytoplasm although glutamate pyruvate transaminase has also been demonstrated in rat liver mitochondria at about 10–30% of the cytoplasmic activity [9,10]. This suggests the possibility that alanine may be transaminated inside mitochondria rather than in the cytoplasm.

Pathways of gluconeogenesis involving the formation of cytoplasmic pyruvate require transport of that pyruvate into mitochondria before carboxylation to oxaloacetate. This pyruvate transport can be blocked by the specific inhibitor α-cyano-4-hydroxycinnamate [11] which has been used in a variety of tissue preparations to specifically inhibit mitochondrial pyruvate metabolism [12]. In this paper we report the effects of tryptophan and α-cyano-4-hydroxycinnamate on gluconeogenesis from a range of substrates in isolated rat and mouse liver parenchymal cells. Our results indicate that serine enters gluconeogenesis at the level of cytoplasmic pyruvate, but that alanine may enter mitochondria before transamination.

2. Materials and methods

Rat and mouse liver parenchymal cells were prepared from starved animals as described previously [13,14] except that in the case of the rat liver cells hyaluronidase was omitted and digestion achieved with the use of collagenase (Type II) alone. For the measurement of the rates of gluconeogenesis, rat liver cells were suspended in medium (Krebs-bicarbonate buffered medium [15] containing 25 mg of albumin/ml) at a concentration of approximately 1.5 mg protein/ml. After incubation at 37°C with continuous gassing (O_2 :CO_2, 95:5) for 30 min in the presence or absence of α-cyano-4-hydroxycinnamate, 1 ml samples of the cell suspension were transferred into plastic incubation vials which were sealed with serum caps and gassed for a further two minutes. Substrates were added by syringe through the serum cap as 100 mM solutions in Krebs-bicarbonate medium

* Permanent address: Laboratório de Física e Engenharia Nucleares, Sacavém, Portugal.
** Present address: Lanchester Polytechnical College, Coventry, UK.
*** Present address: The Biological Laboratory, University of Kent, Canterbury, UK.

to give a final concentration of 10 mM, and incubation continued at 37°C with shaking for 45 min at which time 125 μl of $HClO_4$ (3 M) was added. Denatured protein was removed by centrifugation and 0.7 ml samples of the supernatant were taken for the assay of glucose by the glucose oxidase method [16]. Gluconeogenesis by isolated mouse liver cells was measured similarly but the medium was Hanks medium supplemented with bicarbonate and without added albumin [14]. Oxygen uptake by isolated rat liver cells at 37°C was measured in Krebs-bicarbonate medium containing 25 mg of albumin/ml using a Clark-type oxygen electrode. Cells were present at approximately 4 mg protein/ml. Serine pyruvate transaminase [EC 2.6.1.51] was assayed using glyoxylate reductase [EC 1.1.1.26] as as described by Rowsell et al. [6] using both serine and pyruvate at 10 mM. Protein was determined by a biuret method [17], corrections being made for the albumin present.

Collagenase was obtained from Worthington Biochemical Corp., Freehold, New Jersey, USA, L-(±)-lactate and bovine serine albumin, fraction V, from Sigma Chemical Co. Ltd., London S.W.6 UK, L-amino acids from BDH Chemicals Ltd. Poole, Dorset, UK and all other enzymes and biochemicals from Boehringer Corp. (London) Ltd. London W.5. UK. α-Cyano-4-hydroxycinnamic acid was obtained from Ralph Emmanuel Ltd., Wembley. Middx. HAO 1PY, UK.

3. Results and discussion

The effect of α-cyano-4-hydroxycinnamate on gluconeogenesis from various substrates by isolated rat parenchymal cells is shown in table 1. It is apparent that glucose production from glycerol and glutamine, substrates which do not involve the transport of pyruvate into mitochondria, is not inhibited by α-cyano-4-hydroxycinnamate, whereas that from lactate and pyruvate is substantially inhibited. The inhibition is greater for lactate (92%) than for pyruvate (51%); this may reflect a higher intracellular concentration of pyruvate when the gluconeogenic substrate is pyruvate rather than lactate. It would appear therefore, that α-cyano-4-hydroxycinnamate allows discrimination between gluconeogenic pathways requiring pyruvate transport into mitochondria from those independent of it. Similar results have been reported for gluconeogenesis in kidney cortex slices. [12].

In table 1, we show that under identical experimental conditions gluconeogenesis from serine, but not alanine, was very substantially inhibited by α-cyano-4-hydroxycinnamate. The lack of inhibition of gluconeogenesis from alanine was not due to reversal of the inhibition of pyruvate transport caused by α-cyano-4-hydroxycinnamate, since in the

Table 1
The effect of α-cyano-4-hydroxycinnamate on gluconeogenesis by isolated rat liver cells

Substrate (10 mM)	Rates of glucose formation (nmol/min per mg protein)		Inhibition caused by α-cyano-4-hydroxycinnamate	Number of observations
	Control	With α-cyano-4-hydroxycinnamate (1 mM)	(%)	
Pyruvate	1.29	0.79	51 ± 4	6
Lactate	1.21	0.20	92 ± 2	10
Serine	0.41	0.11	78 ± 5	8
Alanine	0.61	0.58	11 ± 5	6
Glutamine	0.26	0.32	−5 ± 8	8
Glycerol	0.42	0.47	−3 ± 5	8

Isolated rat parenchymal cells were prepared and incubated as described in Materials and methods. Rates of gluconeogenesis are given as the mean of duplicate determinations agreeing within 10% and have been corrected for the rate of glucose production in the absence of substrate (0.12 nmol/min/mg protein) which was unaffected by the presence of inhibitor. The percentage inhibition of gluconeogenesis caused by α-cyano-4-hydroxycinnamate is given as the mean ± S.E.M. of the number of observations shown and involved the use of at least 3 different cell preparations.

presence of lactate and alanine, both at 10 mM, gluconeogenesis was still inhibited to 75 ± 3% (mean ± S.E.M. of 4 observations) of the control rate. Nor is it likely that the inhibition of gluconeogenesis from serine is caused by a decrease in any production of energy from serine oxidation. In the presence of 10 mM serine the rate of oxygen uptake by rat liver cells was 27.4 ng atoms of O_2/min/mg and was unchanged by the addition of 1 mM α-cyano-4-hydroxycinnamate. It would seem likely, therefore, that the energy for gluconeogenesis from serine comes from the oxidation of endogenous fuels such as fatty acids, rather than from the oxidation of serine itself. A further possible explanation of inhibition of gluconeogenesis from serine by α-cyano-4-hydroxycinnamate would be through inhibition of serine pyruvate transaminase. However direct assay of the enzyme in the supernatant fraction of rat liver showed no inhibition on addition of α-cyano-4-hydroxycinnamate (1 mM). The actual amount of enzyme present was also extremely low (19.2 ± 3.4 munits/g wet wt. of liver, mean ± S.E.M. of values from 4 rats) in agreement with other workers [6,8,18], and would appear to be insufficient to account for the observed rates of gluconeogenesis from serine (57.5 nmol/g/min wet wt. of cells).

Hence our results indicate that serine is deaminated to pyruvate by serine dehydratase in the cytoplasm before entering the mitochondria, whereas alanine may enter the mitochondria and be transaminated inside. The conclusion that serine enters gluconeogenesis at the level of pyruvate rather than through hydroxypyruvate is further supported by experiments on isolated mouse liver parenchymal cells (table 2). In these cells, tryptophan produced a marked inhibition of gluconeogenesis from pyruvate, lactate, alanine, propionate and glutamine, but not from glycerol, dihydroxyacetone or fructose. This agrees with the work of Lardy's laboratory showing that tryptophan and its metabolites inhibit gluconeogenesis at the level of phosphoenolpyruvate carboxykinase [7]. However tryptophan also almost totally inhibited gluconeogenesis from serine in mouse liver cells (table 2), again suggesting a route of serine metabolism involving pyruvate rather than hydroxypyruvate. This conclusion agrees with the conventional pathway of serine conversion to glucose involving serine dehydratase. This enzyme shows the expected adaptive changes to diet for an enzyme involved in gluconeogenesis [1,8,19] and would appear to be present in the rat liver at a level sufficient to account for the observed rates of gluconeogenesis.

Alanine is thought to be a major gluconeogenic amino acid and is involved in the transfer of ammonia from the muscle to the liver [20]. Our results suggest that alanine can be transaminated sufficiently

Table 2
The effect of tryptophan on gluconeogenesis by isolated mouse liver cells

Substrate (10 mM)	Rates of glucose formation (nmol/min per mg protein)		Inhibition caused by L-tryptophan
	Control	With L-tryptophan (1 mM)	(%)
Lactate	3.90	0.10	97 ± 1
Serine	2.84	0.70	75 ± 1
Glutamine	2.01	−0.3	>100
Glycerol	4.36	4.64	7 ± 1
Fructose	18.08	17.08	6 ± 1
Alanine	1.14	−0.05	>100
Propionate	0.95	−0.18	>100

Isolated mouse liver cells were prepared and incubated as described in Materials and methods. The concentration of cells was normally about 2.4 mg protein/ml. Rates of gluconeogenesis in the presence of added substrate have been corrected for the rate in the absence of substrate (0.39 nmol/min per mg protein) which was unaffected by the presence of L-tryptophan. Values for the inhibition caused by L-tryptophan are given as the mean ± S.E.M. of three observations.

fast within the mitochondria to account for the observed rates of gluconeogenesis, but they do not necessarily demonstrate that this is the normal site of transamination. The mitochondrial glutamate pyruvate transaminase is very labile [9] and hence accurate assessment of its activity in the liver is difficult. However it would seem to be present at levels sufficient to account for the observed rates of gluconeogenesis and it does show adaptive changes to hormone treatment [10]. Thus an important role for mitochondrial glutamate pyruvate transaminase in gluconeogenesis would seem possible.

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