MITOCHONDRIAL-CYTOSOLIC INTERRELATIONSHIPS INVOLVED IN GLUCONEOGENESIS FROM SERINE IN RAT LIVER

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1. Introduction

In recent years evidence has accumulated suggesting the possibility that mammalian liver may be equipped with alternative pathways for the metabolism of serine towards glucose synthesis. There is good evidence that serine dehydratase (EC 4. 2. 1. 13), which catalyses the formation of pyruvate from serine, is involved in initiating gluconeogenesis in the adult rat (see [1]). Earlier studies suggested the presence of an alternative pathway that involved hydroxypyruvate rather than pyruvate as an intermediate [2] and more recent work with isolated rat liver cells supports this conclusion [3]. Rowsell et al. [4] suggested that this alternative pathway may play a significant role in the suckling neonatal rat and a possible sequence of enzymes to account for this was postulated. The pathway is initiated by serine-pyruvate aminotransferase (EC 2. 6. 1. 51) which catalyses the transamination of serine to form hydroxypyruvate. This enzyme sequence was also proposed by Lardy, et al. [5] to account for gluconeogenesis from serine in the adult rat when the normal pathway via pyruvate was blocked by use of quinolinic acid which inhibits phosphoenolpyruvate carboxykinase (see fig.1). Further evidence for the proposed involvement of the aminotransferase pathway in gluconeogenesis in the neonatal rat was the relative insensitivity of perfused neonatal liver to inhibition of gluconeogenesis from serine by quinolinic acid [6].

For the process of gluconeogenesis in the rat, pyruvate formed by the action of serine dehydratase in the cytosol must be transported into the mitochondria for carboxylation to oxaloacetate to occur (see fig.1). In a recent paper use has been made of the specific inhibitor of pyruvate transport, α-cyano-4-hydroxycinnamic acid, to inhibit the transport of pyruvate and so block the serine dehydratase-mediated pathway of gluconeogenesis from serine [7]. The considerable, though not complete, inhibition of gluconeogenesis from serine in adult rat liver cells in the presence of the inhibitor was taken as evidence for the obligatory participation of pyruvate as an intermediate in gluconeogenesis from serine [7]. The interpretation of of the transport inhibitor for the alternative pathway for serine gluconeogenesis, via serine aminotransferase, requires knowledge of the intracellular site of the initial transamination reaction. The subcellular site has previously been stated to be wholly particulate [8] but supporting data was not given. The present results suggest a predominantly mitochondrial location for the serine aminotransferase activity in adult and neonatal rat liver and it is suggested on this basis that cyanocinnamic acid may not be a suitable inhibitor for discriminating between the different pathways of gluconeogenesis from serine.

2. Methods

Rats were an inbred Wistar albino strain; neonatal animals were suckling 10 day-old rats and adults were male rats of 200–250 g body weight, 5% (w/v) homogenates of liver were gently prepared in 0.4 M-sucrose which contained 0.2 mM-pyridoxal 5′-phosphate. Subcellular fractionation methods were as described by Rowsell et al. [8]. A particulate fraction was prepared by centrifugation of homogenates at 26 700 g for 10 min and resuspended in the homogenizing medium. The supernatant from this centrifugation was taken as

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the cytosol fraction. After sonication, assays were immediately made on the isolated fractions for serine-pyruvate aminotransferase and serine dehydratase [9]. and for subcellular marker enzymes [8].

Biochemicals and auxiliary enzymes used in the assay procedures were from British Drug Houses Ltd., Poole, Dorset, UK and Boehringer Corp. (London) Ltd., London, W.5., UK.

3. Results and discussion

The activities of serine-pyruvate aminotransferase and serine dehydratase in liver homogenates from neonatal and adult rats, together with the subcellular distribution between particulate and cytosol fractions, are given in table 1. Serine aminotransferase was recovered predominantly in the particulate fraction ($\geqslant$ 90%) in both neonatal and adult rat liver. The recovery in both cases was similar to that of glutamate dehydrogenase, a mitochondrial marker enzyme. In contrast, the recovery of serine dehydratase in the particulate fraction was very low and the activity was wholly recovered in the cytosol fraction at both ages. Whole-homogenate activity of serine aminotransferase was higher in neonatal rats compared with adults, whereas serine dehydratase activity was higher in the adult; this is in agreement with previously published work [9].

The particulate fraction from neonatal rat liver was subjected to sucrose density-gradient fractionation to prepare, in separate experiments, a lysosome-enriched fraction ('tritosomes') and a peroxisome-enriched fraction as described in detail by Rowsell, et al. [8]. The lysosomal fraction contained 29–32% (in 3 experiments) of the whole-homogenate acid phosphatase acivity, but only 2–4% of serine aminotransferase acitvity and 2–5% of glutamate dehydrogenase activity. The peroxisomal fraction contained 16–22% (in 3 experiments) of the whole-homogenate urate oxidase activity, but only 4–6% of serine aminotransferase activity and 2–4% of glutamate dehydrogenase activity. The low recovery of serine aminotransferase in comparison to the relevant marker enzyme activity in each if these fractions suggests that neither lysosomes nor peroxisomes are a major subcellular site of the aminotransferase activity. In fact, taken together with the data from differential centrifugation exper-

Table 1
Subcellular distribution of enzyme activities in rat liver fractions

Subcellular fraction	Enzyme activity (μmol/min/g of liver)		
	Serine-pyruvate aminotransferase	Serine dehydratase	Glutamate dehydrogenase
Neonatal			
Whole-homogenate	0.36 ± 0.03	1.18 ± 0.09	41.8 ± 4.4
	(%)	(%)	(%)
Particulate	90 ± 3	6 ± 1	93 ± 3
Cytosol	11 ± 2	108 ± 6	9 ± 2
Adult			
Whole-homogenate	0.14 ± 0.01	3.71 ± 0.94	49.3 ± 3.8
	(%)	(%)	(%)
Particulate	93 ± 3	4 ± 1	92 ± 3
Cytosol	6 ± 2	116 ± 7	5 ± 2

Subcellular fractions were prepared by differential centrifugation of rat liver homogenates as described in Methods. Enzyme activities in the fractions are given as percentages of the whole-homogenate activities. Values are recorded ± S.E.M. for 6 separate experiments.

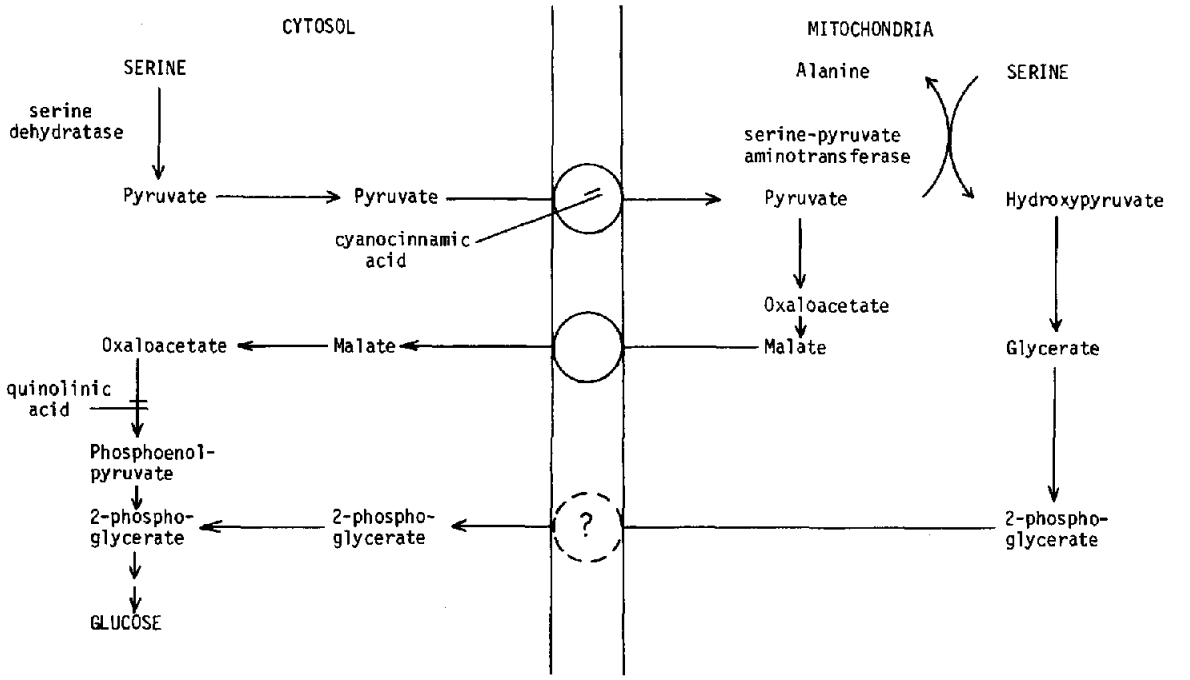

Fig.1. Mitochondrial-cytosolic interrelationships in serine metabolism.

iments (table 1), the results suggest a predominantly mitochondrial location of serine aminotransferase activity.

The localisation of serine dehydratase acivity in the cytosol of neonatal and adult rat liver means that gluconeogenesis from serine involving this pathway will result in the formation of extramitochondrial pyruvate which must be transported into the mitochondria for carboxylation and further metabolism (see fig.1). It is to be expected that inhibitors of pyruvate transport, such as cyanocinnamic acid and its derivatives [10, 11], would be effective in inhibiting carbon flow along this pathway. The alternative gluconeogenic pathway involves an initial transamination of serine to form hydroxypyruvate. If the hydroxypyruvate were formed in the cytosol this would necessitate its transport into the mitochondria for further metabolism to 2-phosphoglycerate (for refs. see [8]). No information on the mitochondrial transport of hydroxypyruvate has been published to date, but in view of the relatively broad specificity of the pyruvate transporter for other monocarboxylates (including hydroxy derivatives) [10,11], one might except that hydroxypyruvate would share this carrier system. In this case inhibition of pyruvate transport would also effectively block hydroxypyruvate transport and prevent metabolism of serine along this pathway. In fact the present results show that there is no necessity for the transport of hydroxypyruvate into mitochondria because the serine aminotransferase activity itself is located intramitochondrially. However, transamination of serine intramitochondrially requires the presence of pyruvate within the mitochondria to act as amino acceptor (see fig.1). There is, of course, no net consumption of pyruvate in this reaction since it can be reformed from alanine by coupled reactions involving alanine aminotransferase and glutamate dehydrogenase. Nevertheless any gross depletion of mitochondrial pyruvate concentrations might be expected to restrict the aminotransferase reaction and decrease carbon flow from serine along the aminotransferase gluconeogenic pathway. In the conditions of the experiments of Mendes-Mourao et al [7], where isolated liver cells were preincubated under $O_2:CO_2$ for 30 min in the presence of the pyruvate transport inhibitor (α-cyano-4-hydroxycinnamate), a considerable re-

duction in the intramitochondrial concentration of pyruvate is to be anticipated [12]. The inhibition of glucose formation from serine upon the subsequent addition of 10 mM-serine as substrate [7] might then be attributed to a block in both the dehydratase and aminotransferase pathways as a result of inhibition of pyruvate transport and depletion of mitochondrial pyruvate concentrations, respectively.

On this basis it is suggested that it is not possible to use inhibitors of pyruvate transport, such as cyanocinnamic acid derivatives, to discriminate between the alternative pathways of gluconeogenesis from serine. On the other hand, as pointed out by Mendes-Mourao, et al. [7], these inhibitors should prove extremely valuable in delineating the role of mitochondrial alanine aminotransferase in gluconeogenesis from alanine, since they would block any contribution by cytosolic alanine aminotransferase to this process. The neonatal rat is of particular interest in this respect because of the much greater proportion of total hepatocellular alanine aminotransferase activity found in the mitochondria compared with adult rats [13] and the possible consequences of this for neonatal nitrogen metabolism [14].

The present finding of a mitochondrial location for serine-pyruvate aminotransferase has further implications with regard to the possible physiological role of this alternative gluconeogenic pathway. It is probable, in the normal fed or fasted adult rat, that the aminotransferase pathway plays a relatively minor role in gluconeogenesis from serine compared with the dehydratase pathway [6, 15, 16]. However, in the case of the neonatal rat it has been suggested that the role of the aminotransferase pathway might be considerably greater, and that the presence of a mitochondrial pathway of gluconeogenesis from serine might be important in terms of a neonatal mechanism for the conservation of the carbon skeleton of hydroxyproline, derived from collagen turnover in the rapidly-growing animal [6, 1].

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