PLASMA-MEMBRANE TRANSPORT OF ALANINE IS RATE-LIMITING FOR ITS METABOLISM IN RAT-LIVER PARENCHYMAL CELLS

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1. Introduction

In the last decade the metabolism of amino acids in liver parenchymal cells has been subjected to intensive research (reviewed in [1]). The presence of specific amino-acid transport systems in the parenchymal cell membrane has been demonstrated, both in whole cells [2–5] and in isolated plasma-membrane vesicles [6–8].

The possible connection between the transport of an amino acid and its subsequent metabolism in the liver cell has so far received only little attention. The uptake of alanine by isolated rat-liver parenchymal cells was reported to be inhibited by ouabain, whereas metabolism of alanine was not affected under these conditions [3]. This observation prompted the conclusion that transport of alanine at the level used (0.5 mM) is not rate-limiting for alanine metabolism [3].

We have investigated the relationship between transport and metabolism of alanine in perifused rat-liver parenchymal cells. The perifusion system [9,10] combines the advantages of the use of homogeneous and purified cell suspensions with those of experimentation under true steady-state conditions at physiologically low substrate concentrations, thus resembling the in vivo situation. Our results, in contrast to those in [3] show that in the physiological concentration range (0.2–0.5 mM) [11,12] the translocation of alanine across the plasma membrane is rate-limiting for alanine metabolism in rat-liver parenchymal cells.

2. Materials and methods

Parenchymal cells were isolated by the method in [13] with minor modifications, from the livers of male Wistar rats (200–300 g), which were either fed ad libitum or fasted for 24 h. Structural integrity of the cells was routinely checked by Trypan blue exclusion. Perifusion of liver cells was performed at 37°C as in [9,10], except that an improved 12 ml perifusion chamber was used with the filter (Sartorius SM 12500) in the upper part of the chamber. Batch incubations of isolated liver cells were performed as in [3] in the presence of 0.5 mM alanine.

Glutamate, α-oxoglutarate and pyruvate were determined using standard enzymic methods [14]. Alanine was determined essentially as in [15]. Before use, the alanine dehydrogenase suspension was centrifuged, resuspended in 20 mM potassium phosphate buffer (pH 7.5) + 1 mM dithiothreitol and dialysed overnight against the same buffer.

Intracellular metabolites in each steady-state condition were determined by removing 0.4 ml samples (containing ~7 mg cells dry wt) from the perifusion chamber, centrifuging the cells through a layer of silicone oil into 14% $HClO_4$ and measuring the metabolites in the neutralized protein-free extract of the cells [16]. An analogous procedure was followed when batch incubations of liver cells were carried out. Discrimination between intra- and extracellular spaces was made by incubating cell samples with [^{14}C]sucrose (spec. act. 0.16 μCi/ml) and ^3H_2O (spec. act. 2 μCi/ml) before silicone oil centrifugation. Under these conditions ~30% of the total pellet volume appeared to be extracellular and the cell volume was 2.27 ± 0.31 μl/mg dry wt (mean ± SEM; n = 5).

3. Results and discussion

Alanine degradation in rat-liver parenchymal cells starts with the transfer of the amino group to α-oxo-

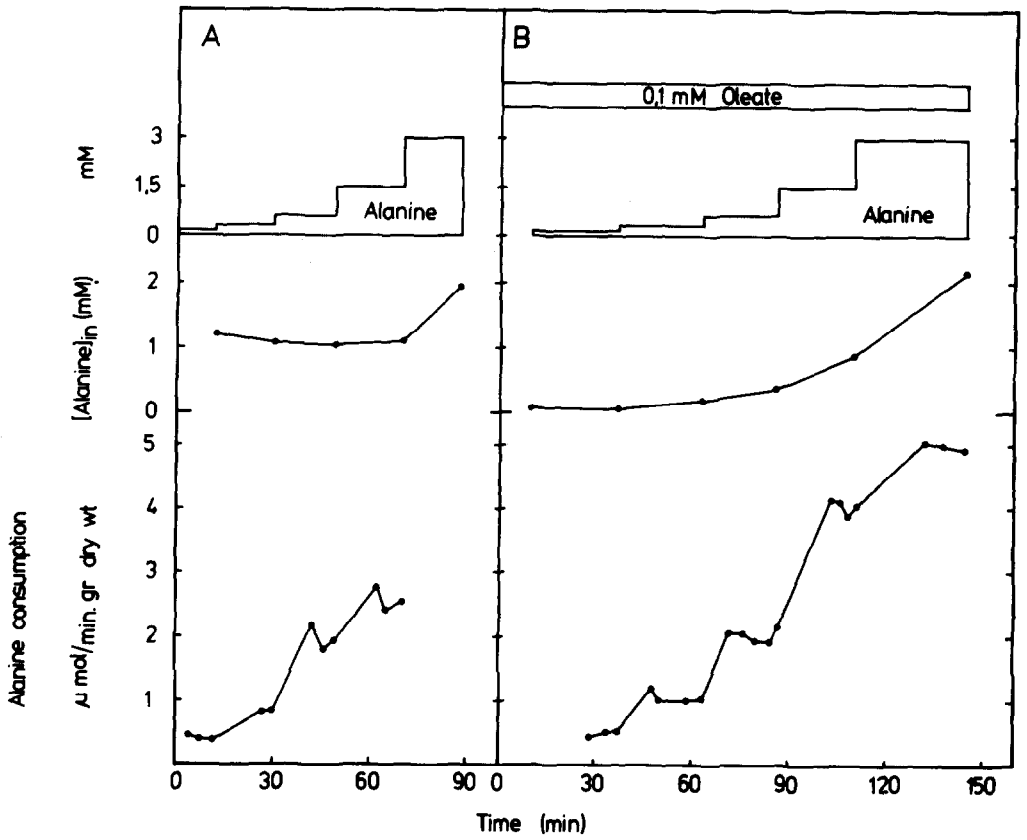

Fig.1. Titrations of perifused rat-liver parenchymal cells with alanine. Cells (~200 mg dry wt) isolated from fed (A) or fasted (B) rats were perifused with alanine (A) or alanine + oleate (B) at the concentrations indicated in the upper part of the figure. Samples (see section 2) were taken at the end of the different steady states for the determination of the intracellular alanine content. Alanine was assayed in the perifusate and alanine consumption was calculated from the difference in alanine concentration in the influent and effluent perifusate. The flow rate was 4 ml/min.

glutarate via glutamate–pyruvate transaminase (EC 2.6.1.2), resulting in the formation of glutamate and pyruvate. Subsequently, glutamate and pyruvate can be converted to urea and glucose, respectively.

In a search for the regulatory step in the catabolic pathway of alanine metabolism, we focussed our attention on the transport of alanine from the external medium into the cell. Fig.1A shows the effect of changes in alanine concentration on the steady-state consumption of the amino acid in perifused liver cells from fed rats. The rate of alanine consumption increased as the concentration of alanine in the influent perifusate was raised. However, no increase was observed in the intracellular alanine concentration except at the highest influent concentration of alanine (3 mM).

The metabolism of alanine in liver parenchymal cells can be inhibited by aminooxyacetate [2,3,16]. The effect of the inhibitor on alanine uptake in perifused liver parenchymal cells from fed rats is shown in table 1 (columns 2,3). Cells of the same batch were divided into two portions and perifused with increasing alanine concentrations either in the presence or in the absence of 0.5 mM aminooxyacetate. In the absence of the inhibitor intracellular alanine was ~1 mM; no increase was observed up to 1.5 mM external alanine (fig.1A). However, when aminooxyacetate was present, the alanine concentration in the cells rose dramatically. These data agree with the observed accumulation of radioactively labelled alanine in rat-liver parenchymal cells in the presence of aminooxyacetate [2–4].

The increase in alanine consumption at an almost

Table 1
Intracellular concentrations of alanine in perifused rat-liver parenchymal cells

Alanine infused (mM)	Intracellular alanine (mM) in rat-liver parenchymal cells			
	Fed state		Fasted state	
	no AOA	+ AOA	no AOA	+ AOA
0	1.0	1.4	0.07	0.9
0.15	1.1	2.4	0.04	2.0
0.3	1.2	3.1	0.2	2.8
0.6	1.1	3.6	0.4	3.6
1.5	1.5	4.9	0.8	5.1
3.0	1.8	n.d.	2.0	6.1
6.0	2.7	7.3	5.1	10.2

Rat-liver parenchymal cells (~210 mg dry wt) were perifused at 37°C with different concentrations of alanine, either in the presence or absence of 0.5 mM aminooxyacetate (AOA). Cell samples were taken at each steady state (fig.1) and the intracellular concentration of alanine was determined as in section 2. The results of 2 expt. are shown, carried out with cells from a fasted and a fed rat, respectively. Similar results were obtained in analogous experiments. n.d., not determined

constant intracellular alanine concentration implies that the glutamate–pyruvate transaminase reaction is in near-equilibrium. It should be emphasized that in a near-equilibrium situation a relatively large change in flux through the enzyme would be accompanied by only a small change in the intracellular alanine concentration [17]. The existence of an almost constant alanine pool of ~1 mM (table 1, [15]) and of high (4–5 mM) intracellular glutamate concentrations (unpublished, [15,18]) is in agreement with a near-equilibrium situation.

Analogous experiments were carried out with liver cells from fasted rats; in these cells the endogenous alanine concentration is very low [19]. As shown in fig.1B, increasing alanine concentrations in the external medium gave rise to increasing alanine consumption rates. The intracellular alanine concentration was low except at 1.5 or 3 mM alanine. However, the intracellular alanine concentration was always lower than the extracellular concentration of alanine. Fractionation of cell samples via the digitonin method [20] indicated that >95% of the alanine in the cells was located in the cytosol both in the presence and absence of aminooxyacetate (not shown). It is clear that under metabolic conditions, alanine does not equilibrate across the plasma membrane (table 1). In liver parenchymal cells from fasted rats, too, addition of aminooxyacetate led to a several-fold accumulation of alanine (table 1, column 5). Measurements of alanine, glutamate, α-oxoglutarate and pyruvate indicate that also in cells from fasted rats, glutamate–pyruvate transaminase operates near equilibrium at the concentrations of alanine used in the perifusion experiments (not shown).

The results in fig.1 and table 1 lead us to conclude that although the transport systems present in the plasma membrane can bring about accumulation of alanine when metabolism of the amino acid is inhibited, no accumulation occurs when alanine can be metabolized. In fact, in cells from fasted rats the intracellular alanine concentration was always lower than that in the perifusate under metabolic conditions.

In view of the results in [3] it was of interest to measure intracellular alanine concentrations in cells incubated batchwise. Isolated parenchymal liver cells from fasted rats were incubated with 0.5 mM alanine for 20 min exactly as in [3]. Alanine consumption, as

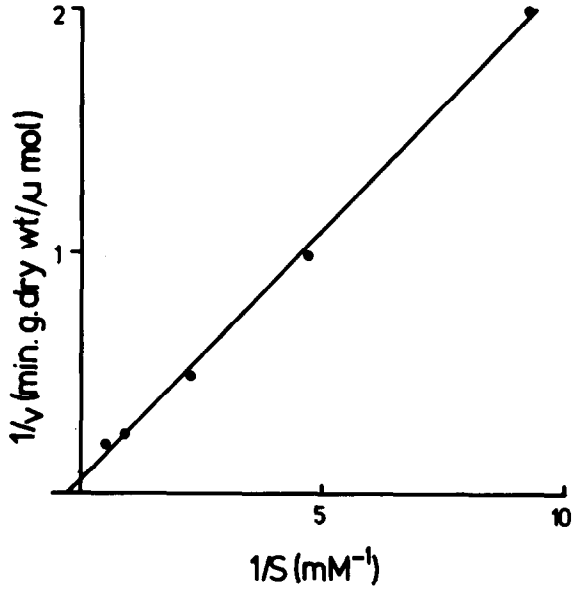

Fig.2. Lineweaver-Burk plot for alanine consumption and the alanine concentration in the perifusate. Isolated rat-liver parenchymal cells from a fasted rat (210 mg dry wt) were perifused with different concentrations of alanine in the presence of 0.1 mM oleate. Consumption of alanine (v) in each steady state was calculated as indicated in fig.1. The concentration of alanine (S) refers to that in the effluent perifusate at each steady state.

measured by the disappearance of alanine from the extracellular medium, was 1.4 μmol . min^{-1} . g dry wt^{-1}, which is comparable to the rate observed in [3]. However, analysis of intracellular metabolites showed that also under these conditions, the intracellular alanine concentration was very low (0.04 mM). Apparently in batchwise incubations, too, transport limits alanine metabolism in the cell. Unfortunately, in [3] there was no report of intracellular alanine concentrations in their experiments.

If transport of alanine is, indeed, the rate-limiting step in the catabolism of this amino acid, one might expect that the kinetics of alanine catabolism would reflect the kinetics of the plasma membrane-bound alanine transport activity. In fig.2 a double reciprocal plot of the steady-state rate of alanine consumption vs external alanine concentration is shown. A straight line is obtained, indicative of simple Michaelis-Menten kinetics. The apparent K_m was 4 mM and the V_{max} was 19 μmol . min^{-1} . g dry wt^{-1}. These parameters agree reasonably well with the kinetics of alanine transport across the cell membrane, when measured under conditions where alanine metabolism is inhibited [3].

We conclude that at physiological alanine concentrations, the transport of alanine across the plasma membrane is rate-limiting for its subsequent catabolism in rat-liver parenchymal cells. It will be of interest to ascertain whether similar considerations will apply to other gluconeogenic or ureogenic amino acids such as asparagine, glutamine, proline and serine.

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