THE TRANSPORT OF BRANCHED-CHAIN AMINO ACIDS INTO ISOLATED RAT LIVER CELLS

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1. Introduction

Isolated liver cells may constitute a useful system for the study of the penetration of metabolites across the liver cell plasma membrane. Hepatocytes isolated from rat liver have been used to study the transport of taurocholic acid [1] and of hexoses [2]. Recently, the uptake of the non-metabolisable amino acid analogues α-aminobutyrate and cycloleucine has been investigated in detail [3,4].

Although the liver is one of the major sites of amino acid metabolism in mammalian systems, relatively little is known about the quantitative characteristics of the transport of naturally-occurring amino acids across the liver cell plasma membrane. The kinetics of amino acid transport can studied in isolated liver cells using radioactive substrates, but a major problem in this approach is the prevention of the metabolism of the labelled compound after it has been transported into the cell. However, it is known that the activity of the branched-chain amino acid transaminase in rat liver is very low [5]. The transport of the branched chain amino acids can therefore be studied in liver cells over short time intervals when no significant breakdown of these compounds will occur.

In this paper, the characteristics of the uptake of L-leucine, L-isoleucine and L-valine into isolated liver cells are described.

2. Experimental

Hepatocytes were prepared from the livers of normally-fed male Wistar rats by the method of Berry and Friend [6] except that hyaluronidase was omitted and the perfusion medium was Ca^{2+}-free Krebs-Henseleit bicarbonate-buffered saline. The cells were stored under oxygen at 0°C at a concentration of 30 mg cell protein/ml in a medium containing 130 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM NaH_2PO_4 and 10 mM Tris 3-(*N*-morpholino)-propanesulphonate at pH 7.4. This medium was also used for the subsequent incubations. The viability of the cells was >90% as judged by Trypan Blue exclusion. Initial rates of transport were measured using a centrifugal filtration technique similar to that used in previous studies [1,2]. 0.05 ml of perchloric acid (15% v/v) was placed in the bottom of a centrifuge tube for the Beckmann Model 152 Centrifuge and 0.1 ml silicone fluid (1:1 v/v dinonylphthalate and Silicone Fluid MS 550 (Hopkins and Williams, Romford, Essex U.K.)) was layered above this. 0.15 ml of incubation medium containing ^{14}C-labelled amino acid and also ^{3}H-labelled inulin formed a layer above the silicone fluid. The cell suspension and contents of the centrifuge tube were equilibrated at the appropriate temperature and the transport reaction was initiated by the addition of 0.1 ml cell suspension

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(3 mg protein) to the top layer. After the appropriate interval, transport was terminated by centrifugation for 5 sec. The supernatant and pellet fractions were assayed for radioactivity as described previously [7]. The uptake of amino acids was taken as the amount of labelled compound appearing in the inulin-impermeable space. We have previously shown [8] that no significant degradation of leucine occurred when liver cells were incubated with 5 mM leucine for periods of up to 1 hour. It is reasonable to conclude that no degradation of leucine occurred in the present experiments which were of much shorter duration.

The ATP content of the cells at 37°C was independent of the length of time that the cells had been previously stored at 0°C. Further, the rate of leucine transport was the same when measured in freshly prepared cells or in cells that had been stored for up to 5 h at 0°C and subsequently warmed to the required temperature.

Cell protein was measured by a biuret method. Radiochemicals were purchased from the Radiochemical Centre, Amersham, U.K.

3. Results

The time course of leucine uptake at 37°C is shown in fig.1(a). The uptake was completed in less than one minute. Extrapolation of the curve to the time axis indicated that the centrifugal filtration method as used here had an effective 'dead time' of 6 sec. This was constant over many experiments and presumably represents the time for which transport continued after the centrifuge was switched on. The uptake of leucine into the inulin-impermeable space exhibited apparent first-order kinetics (fig.1(b)). At 0.5 mM leucine the apparent rate constant was 2.4/min and the rate of leucine uptake was 2.57 nmol/min/mg cell protein. In subsequent experiments, rates were evaluated from measured uptakes at intervals over the first 20 sec using plots similar to that in fig.1(b). The equilibrium uptake was taken as that occurring after 5 min.

The concentration dependence of leucine uptake is shown in fig.2. In agreement with data recently published for the uptake of cycloleucine [4], the initial rate increased linearly with concentration above 10 mM and saturation was not achieved. The probable interpretation of this result is that leucine crosses the membrane via a saturable transport process and also by diffusion. On the assumption that the rate of the non-saturable diffusion component is proportional to concentration over the concentration range used, it is

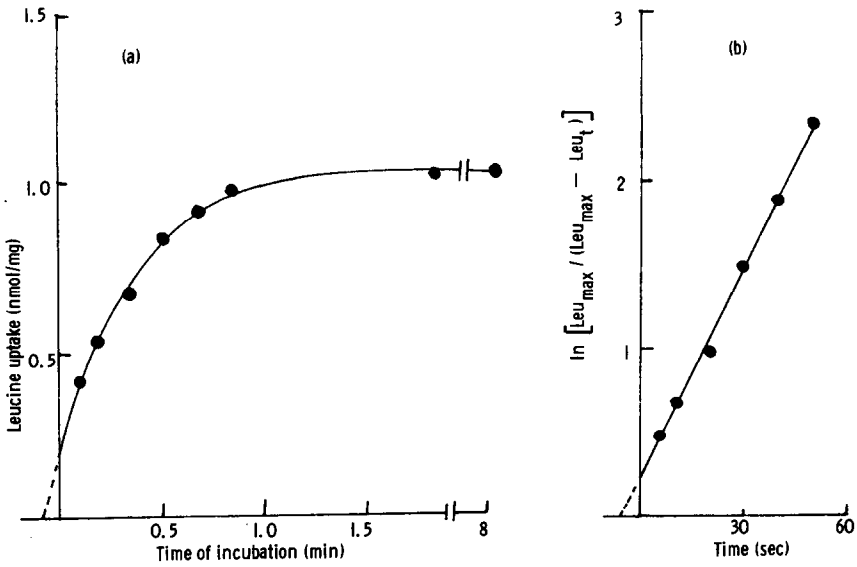

Fig.1.(a) Time course of leucine uptake at 37°C. The leucine concentration was 0.5 mM. (b) First order rate plot of the data in fig.1(a). Leu_{max}: leucine uptake at equilibrium (taken as 1.07 nmol/mg). Leu_t: leucine uptake at time t secs.

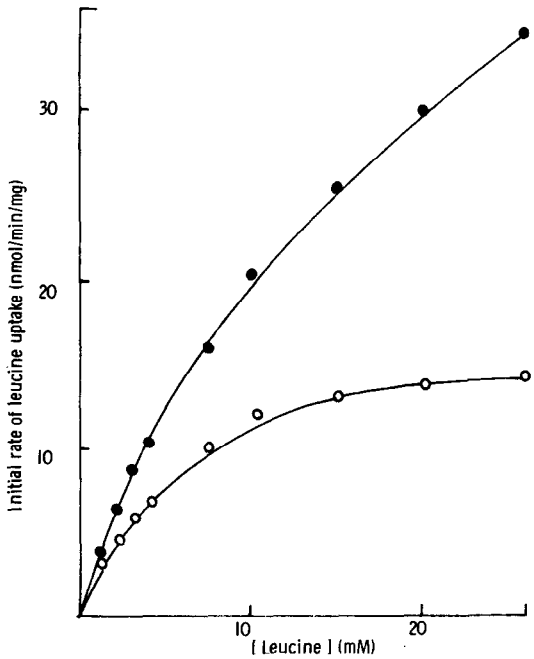

Fig.2. Concentration dependence of leucine uptake at 37°C. (–•—•–): measured rate of leucine uptake. (–○—○–): rate of transport after subtraction of non-saturable component.

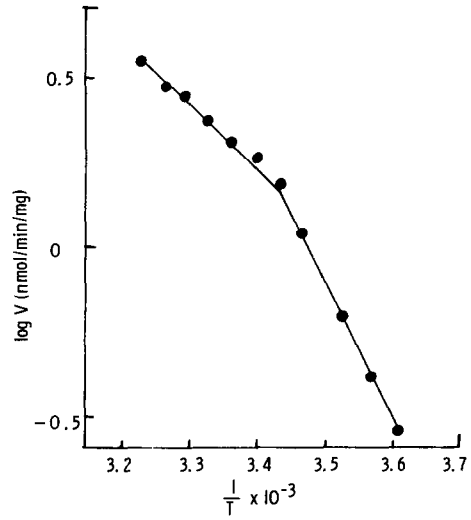

Fig.3. Temperature dependence of leucine uptake. The leucine concentration was 0.5 mM.

possible to subtract the diffusion component from the total uptake. The resulting curve also shown in fig.2. represents the transport of leucine into the cell. The apparent K_m for leucine of this transport process was 5.5 ± 0.6 mM (4 determinations) with a V_{max} of 15.1 ± 2.3 nmol/min/mg at 37°C. The uptake of L-isoleucine and of L-valine showed similar kinetics to that of L-leucine. For isoleucine transport, the apparent K_m was 3.7 ± 0.8 mM and the V_{max} was 13.6 ± 3.2 nmol/min/mg. For valine transport, the apparent K_m was 4.7 ± 0.8 mM with a V_{max} of 13.5 ± 1.0 nmol/min/mg. The ratio [leucine] in inulin impermeable space/[leucine] in medium in the steady state was found to be 1.15 ± 0.21 in determinations on 12 different batches of cells using concentrations of leucine between 0.1 mM and 5.0 mM.

An Arrhenius plot of the temperature dependence of leucine uptake is shown in fig.3. At the concentration used (0.5 mM leucine), the non-saturable component accounted for less than 15% of the rate of uptake at 37°C. The Arrhenius plot showed a discontinuity at 18°C. The apparent activation energy in the range 4–18°C was 80.7 kJ/mole (19.3 kcal/mol) while in the range 18–37°C the apparent activation energy was 32.6 kJ/mol (7.8 kJ/mol). It is of interest that the transport of glucose in isolated hepatocytes shows a similar discontinuity at 18°C (2).

Table 1 shows that the uptake of leucine was inhibited by the simultaneous addition of isoleucine, valine or methionine. Alanine, glycine, serine, threonine and cysteine were without effect. Leucine uptake was also insensitive to ouabain and to the uncoupling agent 2,4-dinitrophenol. In similar experiments (not shown) the uptake of isoleucine was found to be inhibited by leucine and by valine; the uptake o of valine was similarly inhibited by leucine and by isoleucine. Methionine inhibited the uptake of all three branched-chain amino acids to approximately the same extent. In a series of experiments, the rate of transport of any one of the branched-chain amino acids (present at 0.25 mM) was inhibited by each of the other two amino acids (5 mM) by between 25% and 33%. Dixon plots (not shown) established that the uptake of isoleucine was inhibited competitively by leucine (K_I = 4.8 mM). The inhibition of isoleucine uptake by methionine was non-competitive (K_I = 8.7 mM).

Table 1
The effect of various compounds on the uptake of leucine into liver cells

Addition	Leucine uptake after 20 sec (nmol/mg)	% inhibition
None	0.47 ± .01	
L-alanine	0.47 ± .01	
L-glycine	0.46 ± .02	
L-isoleucine	0.34 ± .03[a]	28
L-valine	0.35 ± .03[a]	25
L-serine	0.49 ± .02	
L-threonine	0.45 ± .02	
L-methionine	0.33 ± .04[a]	30
L-cysteine	0.45 ± .02	
Ouabain (0.5 mM)	0.46 ± .02	
2,4 Dinitrophenol (0.5 mM)	0.44 ± .03	

Cells were incubated at 37°C with 0.25 mM L-leucine plus other additions as shown. The amino acids were added at a concentration of 5 mM in each case. Incubations were terminated after 20 sec. The values presented are the mean ± S.E.M. of nine observations.

[a] $p < .01$ V. control

The initial rate of leucine uptake into liver cells was not changed when the incubation medium was replaced by Krebs-Henseleit bicarbonate-buffered saline. Further, the replacement of the NaCl in either medium by an equal concentration of choline chloride had no effect on the initial rate of leucine uptake. The uptake of leucine therefore appeared to be independent of Na^+ or of HCO_3^-; similar results were obtained with respect to the uptake of isoleucine and of valine.

4. Discussion

The kinetics of leucine uptake suggest that at low concentrations this process is mediated primarily by a specific transporting system in the inulin-impermeable cell plasma membrane. This postulate is supported by the strong temperature dependence and by the fact that leucine uptake is inhibited by the other branched-chain amino acids and by methionine. Leucine appears also to penetrate the membrane of isolated hepatocytes by a non-saturable process which represents less than 15% of the initial rate of uptake at the concentrations of leucine which are found in plasma. The uptake of isoleucine and of valine shows similar characteristics to that of leucine and the apparent K_m and V_{max} values for the transport of all three amino acids are similar. The data on the inhibition of the transport of these compounds are consistent with the postulate that leucine, isoleucine and valine are transported with comparable facility on a common carrier system which is non-competitively inhibited by methionine.

Le Cam and Freychet [4] have recently studied the uptake of cycloleucine into isolated liver cells. The uptake of leucine differs from that of cycloleucine in certain respects. Thus leucine uptake is complete in less than 1 min at 37°C while that of cycloleucine continues for 15–20 min at this temperature. The uptake of cycloleucine represents a 3-fold accumulation and is partially dependent on Na^+ ions. However, the branched chain amino acids are not accumulated to any extent, and their uptake is not Na^+ dependent. The present results indicate that at least at low concentrations the transport of the branched chain amino-acids is mediated only by a Na^+-independent system which may have properties similar to the 'L' system identified in Ehrlich ascites cells (see [9]).

In our hands, isolated liver cells incorporate [^{3}H] leucine into acid-precipitable material at a rate of approximately 6 nmol/h/mg cell protein. Half-maximal rate of this process is achieved at 0.2 mM leucine when all the other amino acids are present in excess. If the results obtained on isolated cells reflect accurately the permeability properties of the liver

cell plasma membrane in vivo, it may be concluded that the transport system for branched chain amino acids serves to equilibrate these compounds rapidly across the plasma membrane. The transport of these amino acids is unlikely to limit the rate of protein synthesis in liver.

Acknowledgement

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