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AMINO ACID TRANSPORT SYSTEMS IN THE HUMAN HEPATOMA CELL LINE HEP G2

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Summary: The human hepatoma cell line Hep G2 was used to investigate amino acid transport systems in human liver tissue. The ubiquitous transport systems responsible for the uptake of most neutral amino acids (systems A, ASC and L) were found to be present. Transport system A was predominant for proline uptake but system ASC was the major Na^+-dependent transport system, particularly for glutamine. The specific hepatic system N was functional, but only partially mediated glutamine uptake. The study of Na^+-independent arginine uptake demonstrated the presence of the cationic transport system Y^+, reflecting the transformed nature of Hep G2 cells.

The liver is a major site of amino acid metabolism. Various authors consider that amino acid transport across the plasma membrane of liver cells might be the rate-limiting step in amino acid metabolism (1,2,3). Amino acid transport activity has been extensively investigated in rat liver tissue, but few data have been reported for human liver cells: most have concerned hepatoma cells such as Chang liver cells (4,5), and only one recent study has involved normal liver tissue (6).

The aim of this study was to investigate the transport of selected amino acids by a human hepatoma cell line Hep G2, in which most liver-specific properties are expressed (7,8). We found that ubiquitous transport systems (A, ASC and L) were present, as in rat hepatocytes (9,10). We also sought the presence of the cationic amino acid transport system Y^+, which is a

The abbreviations used are: AIB, α-aminoisobutyric acid; MeAIB, α-(methylamino)isobutyric acid; BCH, β-2-aminobicyclo(2,2,1)heptane-2-carboxylic acid; PBS, Phosphate-buffered saline.

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dedifferentiation marker of normal adult rat hepatocytes (11,12). Particular attention was paid to system N, the Na^+-dependent transport system for glutamine, asparagine and histidine, which is specific to liver tissue and some hepatoma cells of rat origin (13). Finally, we compared the main transport systems in Hep G2 cells with those known to exist in rat hepatocytes.

MATERIALS AND METHODS

Materials:

The radiolabeled amino acids used were α-[1-^{14}C]-methylaminoisobutyric acid (NEN Research Products, France), 2-amino[1-^{14}C]isobutyric acid, L-[U-^{14}C]alanine, L-[U-^{14}C]arginine, L-[U-^{14}C]glutamine, L-[U-^{14}C]histidine, L-[U-^{14}C]leucine, L-[U-^{14}C]proline and L-[U-^{14}C]serine (Dositek, France). Unlabeled amino acids were obtained from Sigma or from Merck. β-2-aminobicyclo(2,2,1)heptane-2-carboxylic acid (BCH) was purchased from Calbiochem-Behring Corp. (CA).

Cell Cultures:

Human hepatoma cells (Hep G2) were grown as monolayers in HAM-F10 medium (Flow Laboratories), pH 7.40, containing 6.1 mM D-glucose, and supplemented with L-glutamine (1 mM), antibiotics (penicillin: 200 U/ml; streptomycin: 200 µg/ml; fungizone: 2.5 µg/ml) and 10% (v/v) fetal calf serum (Flow Laboratories) at 37°C under a humidified atmosphere of 5% CO_2 and 95% air.

Stock cultures of Hep G2 cells were maintained in 75-cm^2 flasks (Falcon Plastics). The culture medium was changed every two days. For transport assays, confluent cells were detached from the flasks with 0.05% (p/v) trypsin plus 0.02% (p/v) EDTA in NaCl solution (Flow Laboratories).

Transport assay:

Cells (1.7 x $10^{5)}$ were seeded into the 2-cm^2 wells of Costar 24-well cluster trays (Costar n°3524) and allowed to grow for 4 days in 1 ml of medium, which was replaced 24 h before experiments. The technique for amino acid transport measurements using the cluster trays was adapted from that described by Gazzola et al (14).

Transport measurements were made in either sodium-containing or sodium-free phosphate-buffered saline solution (Dulbecco's) supplemented with 6 mM D-glucose (PBS-G). The sodium-free medium was prepared by replacing the sodium chloride and sodium hydrogen phosphate buffer with choline chloride and potassium hydrogen phosphate, respectively.

To minimize *trans* effects, cells in culture were washed twice with sodium or choline medium and depleted in 1 ml of the same buffer for 1 h at 37°C. Transport was initiated by adding simultaneously to the wells 0.3 ml of PBS-G containing 0.3 µCi of labeled amino acid plus any other test compounds. Uptake was measured for 1 min at 37°C in a shaking water bath and stopped by pouring off the medium and immediately (<15 sec) washing the wells three times with 2 ml of ice-cold 154 mM sodium or choline chloride. The cluster trays were then drained and the monolayers were solubilized with 0.2 ml of 1 M NaOH by incubation with gentle shaking for at least 2 h at room temperature. From each well, 150 µl of the extract was counted for radioactivity in 10 ml of liquid scintillation counter (Picofluor) and 30 µl was used for protein determination by the method of Lowry et al (15).

Amino acid transport values, expressed as nmol.mg^{-1} of protein.min^{-1}, are the mean ± SD of at least six determinations.

RESULTS AND DISCUSSION

We first measured (Table I) the partition of uptake of natural and artificial amino acids between the Na^+-dependent and Na^+-independent components, using each amino acid as a

Table I. Contribution of Na^+-dependent and Na^+-independent systems to the uptake of several amino acids. Hep G2 cells were incubated for 1 min at 37°C in phosphate-buffered saline solution with indicated labeled amino acid at 0.1 mM. The A and ASC components of amino acid uptake were distinguished by inhibition by MeAIB. The inhibitor to substrate concentration ratio (I/S) was 50. For the different Na -dependent uptakes, the rate in choline-containing buffer was subtracted. The results are the mean ± SD of at least six determinations and are expressed in nmol.mg^{-1} of protein.min^{-1}.

Test amino acid, 0.1 mM	Na^+-dependent uptake		Na^+-independent uptake	Total uptake
	System ASC	System A		
AIB	0.074	0.543	0.069 ± 0.039	0.686 ± 0.062
MeAIB	0.354*	1.03	0	1.38 ± 0.21
L-Serine	5.86	1.82	0	7.68 ± 0.41
L-Alanine	3.38	2.22	0	5.60 ± 0.33
L-Proline	0.462	0.77	0.188 ± 0.005	1.42 ± 0.10
L-Glutamine	4.85	0.90	0.327 ± 0.056	6.08 ± 0.30
L-Histidine	0	0.751	2.66 ± 0.10	3.41 ± 0.24
L-Leucine	0	0	1.80 ± 0.15	1.59 ± 0.03
L-Arginine	0	0.312	0.928 ± 0.056	1.30 ± 0.10

* Uptake in the presence of MeAla instead of MeAIB.

labeled transport substrate. We used MeAIB inhibition to define transport by system A. The part not inhibited by MeAIB was assumed to represent ASC system activity. The Na^+-independent fraction included the nonsaturable components, as well as transport systems L and/or Y^+.

At extracellular substrate concentrations of 0.1 mM, the non-metabolizable alanine analogs AIB and MeAIB were essentially transported by system A. For AIB transport, an Na^+-independent route was present, representing about 10 per cent. This component of AIB uptake may represent either passive diffusion or the contribution of system L. The same feature has been described in rat hepatocytes (16). In Hep G2 cells, despite weak transport activity which escaped inhibition by the methylated analogs, MeAIB was the most specific substrate for transport system A.

Transport of alanine and serine was Na^+-dependent, whereas a portion of proline uptake occurred via Na^+-independent transport systems. Again at extracellular substrate concentrations of 0.1 mM, the partition of these amino acids between systems A and ASC showed the absence of a specific substrate for system ASC. Threonine has been employed as a reliable indicator of the activity of system ASC, but in human fibroblasts (17). In rat hepatocytes, threonine has been used as inhibitor of both system A and system ASC (18). The lack of a model analog of

system ASC that can be used to inhibit this system efficiently is an important problem in studies with many cell types (19,20).

At 0.1 mM, we found that glutamine transport was partitioned among at least three systems: one Na^+-independent route and two Na^+-dependent routes (systems A and ASC). The apparently high degree of ASC transport shown by this amino acid is an interesting feature in this cell type. System ASC, defined as Na^+-dependent transport that escapes MeAIB inhibition, might include another transport system such as system N (13).

Transport of histidine, leucine and arginine is essentially (histidine and arginine) or completely (leucine) Na^+-independent. The weak Na^+-dependent transport of histidine and arginine is due to system A, as shown by the complete MeAIB inhibition.

This partition of transport of these amino acids between distinct transport systems occurred at 0.1 mM. For each amino acid, we investigated the relative contributions of each transport system at different extracellular substrate concentrations.

Fig. 1 represents the respective activities of systems A and ASC for alanine, serine and proline at three extracellular concentrations (0.01, 0.1 and 1 mM).

At 0.01 mM, alanine and serine transport escaped system A activity, with system ASC being the major route. In contrast, at the same extracellular concentration, system A was

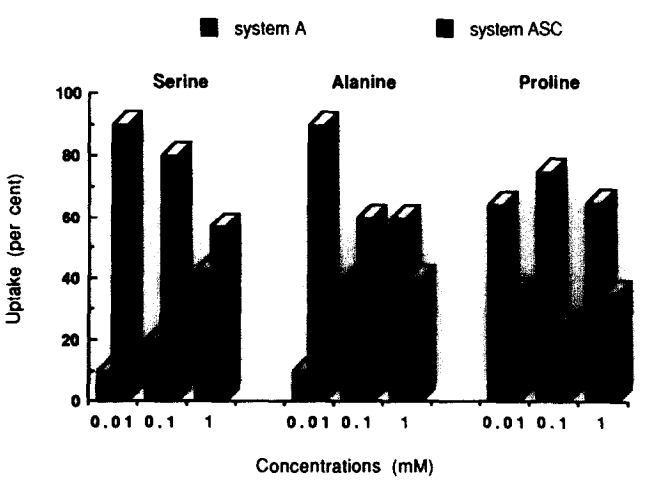

Figure 1. Respective roles of system A and system ASC in alanine, serine and proline uptake at various extracellular concentrations. Uptake of the selected amino acid at three extracellular concentrations was measured for 1 min at 37°C in the presence of sodium, and the rate in choline-containing buffer was subtracted. System A and system ASC were distinguished by using MeAIB inhibition. Furthermore, Na^+-dependent uptake was completely abolished in the presence of MeAIB plus threonine and MeAIB plus serine.

predominant for proline transport (≥ 65% of total Na^+-dependent transport). With increasing extracellular concentrations, no change occurred for proline uptake, whereas the role played by system A became significant for alanine and serine. Kilberg et al showed that in rat hepatocytes at least two transport systems (A and ASC) participated in the Na^+-dependent uptake of serine (10). This dual intervention exists in normal cells but also in some transformed cells (20). Moreover, as in rat hepatocytes (21,22), system ASC presented a higher velocity than system A for serine and alanine in Hep G2 cells. In contrast, Mailliard and Kilberg showed that alanine was almost exclusively transported by system A in plasma membrane vesicles isolated from normal human liver (6).

Table II shows the Na^+-independent transport of leucine and arginine. Na^+-independent leucine transport was totally inhibited by BCH, a specific inhibitor of transport system L, at extracellular substrate concentrations of 0.01, 0.1 and 1 mM (data not shown). In contrast, complete inhibition of arginine transport occurred only in the presence of lysine and homoarginine, specific substrates for system Y^+. Thus, system Y^+ is significant in Hep G2 cells, as in fetal rat hepatocytes and rat hepatoma cells (11,12). White and Christensen suggested that the appearance of system Y^+, associated with the loss of arginase in rat hepatoma cells, may be a critical step in liver cell transformation (12). Our results suggest similar differences between normal and transformed human liver cells.

Table II. L-leucine and L-arginine uptake into Hep G2 cells in the presence of natural and artificial amino acids. Uptake of the labeled test amino acid at 0.1 mM was measured for 1 min at 37°C in the presence or absence of 5 mM inhibitor. The data are the means of at least six determinations and are expressed as the per cent of total uptake in Na^+-containing buffer and in the absence of inhibitor (100 per cent uptake). The 100 per cent control rates are given in parentheses as nmol.mg^{-1} of protein.min^{-1}.

	Per cent of uptake	
Inhibitor, 5 mM	**0.1 mM L-Leucine**	**0.1 mM L-Arginine**
None	100 (1.59 ± 0.03)	100 (1.30 ± 0.10)
MeAIB	92	79
L-Proline	72	ND
BCH	0	75
L-Phenylalanine	4	ND
L-Histidine	7	4
L-Lysine	70	0
L-Homoarginine	68	0

As shown in Table I, glutamine transport was essentially Na^+-dependent. The point we focused on was the major Na^+-dependent route not inhibited by MeAIB (Fig. 2). Serine and threonine were good inhibitors of glutamine transport. If only systems A and ASC were responsible for glutamine transport, inhibition by MeAIB plus threonine (or by serine) should be complete. However, about 20 per cent of glutamine transport escaped inhibition by MeAIB plus threonine. Histidine or histidine plus MeAIB decreased glutamine transport to a lesser extent. Glutamine transport was completely abolished by histidine plus threonine. Our results suggest the contribution of a third Na^+-dependent route, possibly system N. Vadgama and Christensen have shown that glutamine is an effective model substrate for system N in fetal and mature rat hepatocytes and in rat hepatoma cells (H35), whereas histidine is a poor substrate but a good inhibitor of this system when glutamine is used as the test substrate, except in fetal hepatocytes (23).

To confirm the presence of system N, we used another specific property of system N, i.e. its tolerance of lithium substitution for sodium ions (Table III). In human Hep G2 cells, in the presence of lithium and the absence of an inhibitor, Na^+-dependent, Li^+-tolerant glutamine

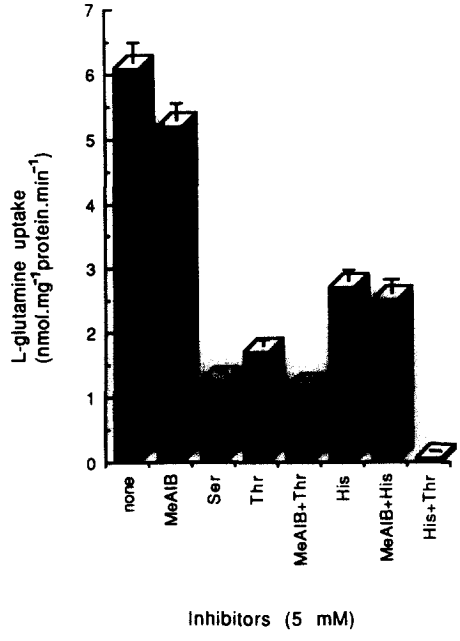

Figure 2. Inhibition of Na^+-dependent uptake of L-glutamine in Hep G2 cells. Sodium-dependent uptake of labeled 0.1 mM L-glutamine was measured for 1 min at 37°C in the presence or absence of 5 mM of one or two inhibitors. The results are the mean ± SD of at least six determinations and are expressed in nmol.mg^{-1} of protein.min^{-1}.

Table III. Comparative effect of Na^+ and Li^+ on L-glutamine uptake into Hep G2 cells. Uptake of labeled 0.1 mM L-glutamine into Hep G2 cells was measured for 1 min at 37 °C in the presence of sodium or lithium. The lithium medium was prepared by replacing sodium chloride with lithium chloride. The results are expressed as the per cent of total uptake in Na^+-containing buffer and in the absence of inhibitor (100 per cent uptake).

Cation present during assay	Inhibitors	0.1 mM L-Glutamine per cent of uptake
Na^+	None	100
Na^+	MeAIB	86
Li^+	None	23
Li^+	L-Histidine	0

transport persisted. However, this part of glutamine transport at an extracellular concentration of 0.1 mM was minor, while in both fetal and adult rat hepatocytes glutamine transport is virtually restricted to system N (13,23).

Table IV shows the different transport system components responsible for glutamine uptake at various extracellular concentrations. System A transport activity increased significantly with the extracellular concentration of glutamine, while system ASC was predominant at 0.01 and 0.1 mM. Glutamine transport by system N was weak but always present. There was neither an enhancement nor an inhibition of system N activity with increasing extracellular substrate concentrations.

In conclusion, we found that several amino acid transport systems analogous to those described in the rat liver were present in Hep G2 cells, yet with significant differences. System Y^+, barely detectable in isolated hepatocytes but present in transformed liver cells of rat origin

Table IV. Respective roles of amino acid transport systems in L-glutamine uptake at various extracellular concentrations. Uptake of labeled L-glutamine at three extracellular concentration was measured for 1 min at 37°C. For system L and system A, we used respectively BCH-inhibition and MeAIB-inhibition, in the presence of sodium. System N activity was measured by glutamine uptake in the presence of lithium. System ASC was only estimated subtracting the previous rates.

Transport system tested	per cent of uptake		
	0.01 mM L-Glutamine	0.1 mM L-Glutamine	1 mM L-Glutamine
A	5	15	34
ASC	77	62	30
N	15	16	22
L	3	7	14

(12,23), played a significant role in Hep G2 cells for arginine transport. System ASC was the major Na^+-dependent transport system in Hep G2 cells, as in rat hepatocytes, even for glutamine transport. In rat liver, system N is predominant for glutamine transport, while in Hep G2 cells it was functional but not predominant. This is similar to the situation in human liver plasma membrane vesicles (6). The weak activity of system N we observed might be specific to human tissue. Although Hep G2 cells are transformed cells, they retain a number of normal hepatocyte functions and are a useful tool for transport studies of human liver.

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