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Further studies on amino acid transport in murine P388 leukemia cells in vitro. Presence of system y^+

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The transport of glycine and L-lysine into murine P388 leukemia cells has been examined. Glycine transport appears to be shared by both systems A and ASC in P388 cells. Glycine transport is Na^+-dependent and is effectively blocked by α-(methylamino)isobutyric acid, threonine and alanine but only a marginal reduction in transport is seen with 100-fold excess cold 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid. System gly is not expressed in P388 cells. Lysine is largely transported by a Na^+-independent, pH-insensitive system with a K_m of 0.079 mM. Lysine transport is relatively unaffected by the addition of 100-fold excess cold α-(methylamino)isobutyric acid, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid and the anionic amino acids, L-glutamate and L-aspartate. A partial inhibition of lysine transport was observed with L-threonine and L-leucine while L-arginine and L-histidine radically decreased lysine transport. Lysine appears to be transported by a system similar to the system y^+ seen in cultured human fibroblasts, Ehrlich ascites cells, and hepatoma cell lines.

Introduction

Amino acid transport has been studied in a variety of cell types, and a number of amino acid transport systems have been identified. Systems L, A and ASC appear to be ubiquitous, but other less common transport systems have been reported in selected cell lines. Our previous investigations on the amino acid transport systems in P388 murine leukemia cells verified the presence of systems L, A and ASC; as well, the existence of a system N was established [1]. System N had previously been reported in rat hepatocytes and hepatoma cell lines [2,3]. Other less common transport systems include a glycine-specific (system gly) demonstrated in pigeon erythrocytes [4], rat hepatocytes [5], and Ehrlich cells [6]. Separate systems for charged amino acids have also been shown. Cationic amino acids are transported by system y^+ as seen in Ehrlich ascites cells [7], cultured human fibroblasts [8] and hepatoma cells [9], while anionic amino acids are transported by two separate systems in hepatoma cells (system X_A and X_G) [10,11] and rat hepatocytes [11].

We have extended our studies on amino acid transport in P388 cells. The transport of L-lysine, as representative of the cationic amino acids, and glycine have been examined. In this paper, we

Abbreviation: BCH, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid.

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report on the existence of system y^+ and the lack of a separate transport system specific for glycine in P388 cells.

Materials and Methods

Materials

L-[U-^{14}C]Lysine (348 mCi/mmol), [1-^{14}C] glycine (56 mCi/mmol), [*carboxyl*-^{14}C]inulin (3.2 mCi/mmol) and tritiated water (90 μCi/mmol) were all purchased from Amersham Corporation. α-[Methylamino]isobutyric acid and the unlabelled amino acids were purchased from Sigma Chemical Co. 2-Aminobicyclo[2,2,1]heptane-2-carboxylic acid (BCH, isomeric form b($\pm$)) was purchased from Calbiochem-Behring Corp. Dulbecco's phosphate-buffered saline was supplied by Gibco; bovine serum albumin (Fraction V, powder, low-salt and salt-free fractions) from Miles Laboratories; Versilube F-50 silicone oil from Nessa Products; choline chloride, dextrose and other chemicals from Fisher Laboratories.

Harvesting of P388 leukemia cells

Male BALB/c $\times$DBA/2F mice (hereafter called CD2F$_1$ mice) weighing 17–25 g were maintained on LAB-BLOX laboratory chow pellets and water ad libitum were used throughout this study. CD2F$_1$ mice were implanted intraperitoneally with $2 \cdot 10^6$ murine P388 leukemia cells. The mice were killed by cervical traction 4–5 days after implantation, and the P388 cells were aspirated from their abdomens. The P388 cells were suspended in either a Na^+-rich medium containing phosphate-buffered saline (pH 7.4), 0.25% dextrose, and 0.1 mM low-salt bovine serum albumin, or a Na^+-free choline-substituted medium containing 0.1% dextrose and 0.1 mM salt-free bovine serum albumin as described previously [1,12]. The cells were then washed twice in the appropriate medium with centrifugations at $200 \times g$ for 5 min at 23°C. All final cell suspensions were in their appropriate medium at a concentration of $4 \cdot 10^6$ P388 cells/ml.

Cell viability was typically 95% by Trypan blue exclusion. The percentage of red blood cells in P388 cell suspensions was always $\leq 5\%$.

Transport experiments

Prior to the initiation of transport experiments, all cell suspensions and amino acid solutions were pre-incubated for 5 min at 37°C in a shaking water bath. Transport was initiated by the addition of a labelled amino acid in the appropriate medium to an equivalent volume of suspended P388 cells ($4 \cdot 10^6$ cells/ml) at 37°C. At indicated time points, 400-μl aliquots of the amino acid-P388 cell incubation mixture were layered onto 1 ml of Versilube F-50 silicone oil in microcentrifuge tubes in duplicate or triplicate. The assay was terminated by centrifugation of the samples at $12000 \times g$ for 1 min at 23°C in a Brinkmann Eppendorf microcentrifuge to separate cells from medium as previously described [1,13]. The supernatants were discarded and the cell pellets were solubilized in 100 μl of 0.6 M perchloric acid overnight at 4°C. The tubes were then centrifuged for 3 min at $12000 \times g$ at 23°C to pellet cellular macromolecules. The supernatants were removed and placed in scintillation vials containing 10 ml scintillation cocktail. The radioactivity was determined via scintillation counting in a Wallac 1217 Rackbeta liquid scintillation spectrophotometer and corrected for quenching.

The intracellular water space (ICW) was determined with tritiated water plus [*carboxyl*-^{14}C]inulin for all transport studies by centrifugation through Versilube F-50 silicone oil to separate medium from cells as previously described [14].

Calculations

Kinetic parameters were determined by linear regression analysis and were equivalent when plotted on either Lineweaver-Burk or Eadie-Hofstee plots. All uptake data are expressed in nanomoles of tested amino acid per μl of intracellular water.

Results

Glycine transport

Fig. 1 shows the Na^+-dependent uptake of 0.05 mM glycine by P388 leukemia cells at 37°C. The uptake of glycine was rapid at early incubation times but had not reached steady-state levels following a 10 min incubation. The Na^+-independent uptake of glycine was 8% of total uptake at 20 s and was always $< 12\%$ of total uptake.

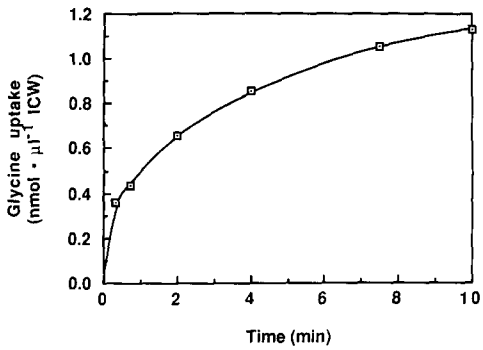

Fig. 1. Time-course of 0.05 mM glycine by P388 leukemia cells in suspension. The cells were incubated in Na^+-rich and choline-substituted media with 0.05 mM labelled glycine at 37 °C for a period of up to 10 min, with aliquots removed from the incubation mixture at specified time points (see Materials and Methods). The data shown are the Na^+-dependent rates only.

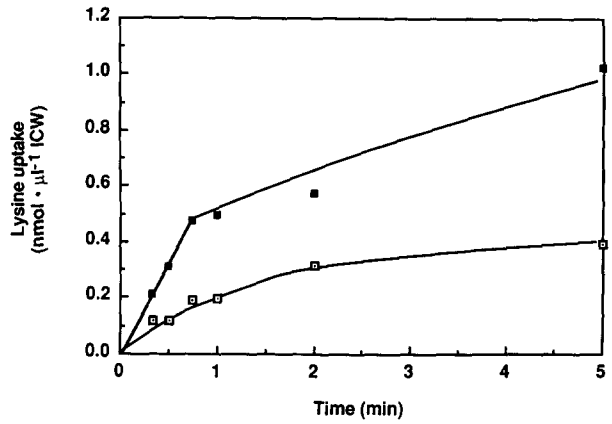

Fig. 2. Time-course of 0.05 mM or 1.0 mM L-lysine by P388 leukemia cells in suspension. The cells were incubated in Na^+-rich media with 0.05 or 1.0 mM labelled L-lysine at 37 °C for a period of up to 5 min, with aliquots removed from the incubation mixture at specified time points (see Materials and Methods), (□) 0.05 mM L-[^{14}C]lysine; (■) 1.0 mM L-[^{14}C]lysine.

The uptake of 0.05 mM glycine was tested for possible inhibition by a 100-fold excess of various amino acids and amino acid analogues. Table I shows that both BCH, a non-metabolizable amino acid analogue known to enter cells by the Na^+-independent system L, and glutamine, shown to be transported by both systems ASC and N in P388 cells [1], partially reduced glycine transport in P388 cells. α-(Methylamino)isobutyric acid, a non-metabolizable amino acid analogue known to enter cells specifically by the Na^+-dependent system A, decreased glycine transport by > 70%. As well, threonine, shown to be a specific substrate for the Na^+-dependent transport system ASC in P388 cells [1], inhibited glycine transport by 62%. Alanine, which enters cells by both systems A and ASC, inhibited glycine transport almost as effectively as 100-fold excess cold glycine (21% versus 17%).

TABLE I

INHIBITION OF GLYCINE UPTAKE BY A VARIETY OF NATURAL AND ARTIFICIAL AMINO ACIDS

Transport of 0.1 mM labelled glycine into P388 leukemia cells in suspension was measured in the presence or absence of 5 mM unlabeled inhibitor. The uptakes were for 30 s at 37 °C as described under Materials and Methods. The data are expressed as the mean ± S.E. for three to six determinations. The uninhibited velocity in Na^+-rich media was 1.56 ± 0.46 nmol $(\mu l\ ICW)^{-1} \cdot min^{-1}$. MeAIB, α-(methylamino)isobutyric acid.

Inhibitor tested (5 mM)	Uptake (% of control)
None	100
BCH	53 ± 4
MeAIB	28 ± 7
L-Threonine	38 ± 9
L-Glutamine	62 ± 6
L-Alanine	21 ± 3
Glycine	17 ± 3
In choline-substituted media (− inhibitor)	7 ± 1

TABLE II

UPTAKE OF L-LYSINE IN Na^+-RICH OR CHOLINE-SUBSTITUTED MEDIA

Transport of 0.1 mM labelled L-lysine into P388 leukemia cells in Na^+-rich or choline-substituted (i.e., Na^+-free) media. The uptakes were at 37 °C for a period of up to 7.5 min, with aliquots removed from the incubation mixture at specified time points (see Materials and Methods). The data are expressed as the mean ± S.E. for two determinations.

Time (min)	Uptake (nmol · (µl ICW)$^{-1}$)		Uptake in choline-media (%)
	Na^+-rich media	Choline-substituted media	Uptake in Na^+-media
0.25	0.32 ± 0.01	0.21 ± 0.01	56 ± 6
0.75	0.40 ± 0.06	0.31 ± 0.02	78 ± 6
2.0	0.56 ± 0.03	0.49 ± 0.02	88 ± 12
4.0	0.79 ± 0.03	0.72 ± 0.01	93 ± 8
7.5	0.98 ± 0.04	1.05 ± 0.01	> 100

The uptake of 0.05 and 1.0 mM L-lysine by P388 leukemia cells was linear up to 45 s in Na^+-rich media (Fig. 2). Lysine uptake approached steady-state levels after a 5 min incubation time at both 0.05 and 1.0 mM. In choline-substituted media (Table II), the uptake of 0.1 mM lysine was reduced by 35% as compared to uptake in Na^+-rich media after 15 s of incubation. This reduction disappeared as the duration of the incubation increased. The observed K_m for Na^+-independent lysine transport into P388 cells was 0.079 ± 0.011 mM (mean ± S.E., $n = 5$) while the V_{max} was 1.37 ± 0.28 nmol·(μl ICW)$^{-1}$·min^{-1} (mean ± S.E., $n = 5$).

Table III shows that α-(methylamino)isobutyric acid showed little inhibition of lysine transport. Threonine inhibited lysine transport by approx. 30% while lysine transport was only marginally inhibited by BCH and the anionic amino acids. Lysine uptake was inhibited to 64% of control by leucine, which appears to be transported by systems L, A and ASC in P388 cells (unpublished results). The most effective inhibitors of lysine transport were the cationic amino acids, histidine and arginine, which reduced lysine uptake to 33 and 18% of control, respectively.

TABLE III

INHIBITION OF L-LYSINE UPTAKE BY A VARIETY OF NATURAL AND ARTIFICIAL AMINO ACIDS

Transport of 0.1 mM labelled L-lysine into P388 leukemia cells in suspension was measured in the presence or absence of 10 mM unlabelled inhibitor. The uptakes were for 30 s at 37°C as described under Materials and Methods. The data are expressed as the mean ± S.E. for three to five determinations. The uninhibited velocity in Na^+-rich media was 1.36 ± 26 nmol·(μl ICW)$^{-1}$·min^{-1}. MeAIB, α-(methylamino)isobutyric acid.

Inhibitor tested (10 mM)	Uptake (% of control)
None	100
BCH	87 ± 9
MeAIB	94 ± 11
L-Threonine	71 ± 6
L-Leucine	64 ± 5
xl-Arginine	18 ± 2
L-Histidine	33 ± 2
L-Glutamic acid	88 ± 7
L-Aspartic acid	84 ± 10
L-Lysine	18 ± 3
In choline-sbustituted media (− inhibitor)	74 ± 6

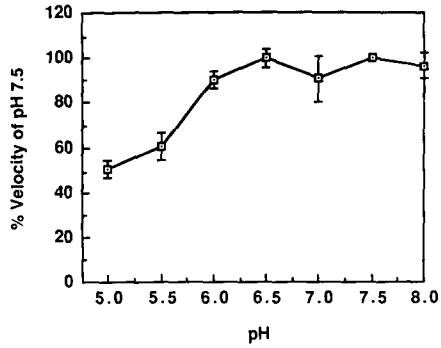

Fig. 3. The relation between pH of the uptake medium and the uptake of labelled L-lysine in P388 leukemia cells. P388 leukemia cells in suspension were assayed at varying pH values between 5.0 and 8.0. The data presented are rates for the Na^+-independent component only at each of the pH values examined. The rate was 0.53 ± 0.02 nmol·(μl ICW)$^{-1}$·min^{-1} at pH 7.5. Uptakes were measured for 30 s incubations at 37°C as described under Materials and Methods. The data are the means ± S.E. of three determinations.

The uptake rates of lysine into P388 leukemia cells between pH 5.0 and pH 8.0 in choline-substituted media are shown in Fig. 3. The uptake of 0.1 mM lysine was not significantly affected by a raise in the $[H^+]$ from pH 8.0 to pH 6.0. Lysine transport showed pH sensitivity at the lower pH values of 5.0 and 5.5.

Discussion

Glycine has been shown to be transported by a specific transport system (system gly) in pigeon erythrocytes [4] and Ehrlich cells [6]. System gly has been shown to be present in hepatoma cell lines and in the ordinary rat hepatocyte [5], but glycine is also transported by other systems in these cells. Results of the present study indicate that glycine uptake in P388 leukemia cells is mediated by the two ubiquitous amino acid transport systems – systems A and ASC. Glycine uptake was effectively blocked by α-(methylamino)isobutyric acid, a specific inhibitor of amino acid transport system A; alanine, which is transported by both systems A and ASC in P388 cells; and threonine, a specific inhibitor of system ASC [1]. These results suggest that glycine enters P388 cells

by both systems A and ASC. Furthermore, only 7% of glycine transport is Na^+-independent after a 30 s incubation, suggesting that little or no glycine uptake into P388 cells is via the Na^+-independent system L. The partial inhibition expressed by BCH may be attributed to non-competitive interference with glycine transport. These results suggest that glycine does not appear to be transported by a glycine-specific system such as system gly in P388 cells.

The inhibition of lysine transport into P388 cells showed a different pattern as compared to that of glycine. None of the amino acids or amino acid analogues specific for any of the major amino acid transport systems blocked lysine uptake to any substantial degree. The partial decrease in lysine transport observed with 100-fold excess unlabelled threonine suggests that the Na^+-dependent portion of lysine transport appears to be via system ASC. Lysine uptake appears, however, to become totally Na^+-independent as the duration of the incubation increases. The only effective blockers of lysine transport were the basic amino acids, arginine and histidine, suggesting that lysine is largely transported into P388 cells by a Na^+-independent transport system specific for cationic amino acids. The observed K_m for this system of 79 μM is similar to reported values in hepatoma cells [9] and cultured human fibroblasts [8] and slightly lower than those reported in intestinal or renal brush-border membranes [15,16]. Lysine transport was not suppressed by increasing the $[H^+]$ from pH 8.0 to pH 6.0. This is similar to the pH-insensitive lysine influx in brush-border membranes [16] and intestinal epithelial cells [17], and the pH-insensitive arginine influx in cultured human fibroblasts [8] and hepatoma cells [9] over the pH range 5.5–8.0. These results suggest that lysine is transported into P388 cells by a specific system similar to system y^+.

In conclusion, system gly does not appear to be present in P388 cells. Glycine appears to be transported largely by systems A and ASC. Lysine, on the other hand, is largely transported by a Na^+-independent, pH-insensitive system that appears to be specific for cationic amino acids. This system appears to be similar to the system y^+ of cultured human fibroblasts [8] and hepatoma cells [9].

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References

1 Lazarus, P. and Panasci, L.C. (1986) Biochim. Biophys. Acta 856, 488–495
2 Kilberg, M.S., Handlogten, M.E. and Christensen, H.N. (1980) J. Biol. Chem. 255, 4011–4019
3 Kilberg, M.S. (1982) J. Membrane Biol. 69, 1–12
4 Vidaver, G.A. (1964) Biochemistry 3, 662–667
5 Christensen, H.N. and Handlogten, M.E. (1981) Biochem. Biophys. Res. Commun. 98, 102–107
6 Christensen, H.N. (1964) J. Biol. Chem. 239, 3584–3589
7 Christensen, H.N., Handlogten, M.E. and Thomas, E.L. (1966) Proc. Natl. Acad. Sci. USA 63, 948–955
8 White, M.F., Gazzola, G.C. and Christensen, H.N. (1982) J. Biol. Chem. 257, 4443–4449
9 White, M.F. and Christensen, H.N. (1982) J. Biol. Chem. 257, 4450–4457
10 Gazzola, G.C., Dall'Asta, V., Bussolati, O., Makowske, M. and Christensen, H.N. (1981) J. Biol. Chem. 256, 6054–6059
11 Makowske, M. and Christensen, H.N. (1982) J. Biol. Chem. 257, 5663–5670
12 Vistica, D.T. and Schuette, B.P. (1981) Mol. Pharmacol. 19, 92–96
13 Vistica, D.T. and Rabinovitz, M. (1979) Biochem. Biophys. Res. Commun. 86, 929–932
14 Wohlhueter, R.M., Marz, R., Graff, J.C. and Plagemann, P.G.W. (1976) J. Cell. Physiol. 89, 605–612
15 Stieger, G., Strange, G., Biber, S.J. and Murer, H. (1983) Pflügers Arch. 397, 106–113
16 Cassano, G., Leszczynska, B. and Murer, H. (1983) Pflügers Arch. 397, 114–120
17 Reiser, S. and Christiansen, P.A. (1973) Biochim. Biophys. Acta 307, 223–233