Characterization of L-glutamine transport by a human neuroblastoma cell line

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Wasa, Masafumi, Hong-Sheng Wang, and Akira Okada. Characterization of L-glutamine transport by a human neuroblastoma cell line. Am J Physiol Cell Physiol 282: C1246–C1253, 2002. First published January 9, 2002; 10.1152/ajpcell.00324.2001.—This study characterized the Na⁺-dependent transport of L-glutamine by a human neuroblastoma cell line, SK-N-SH. The Na⁺-dependent component represented >95% of the total glutamine uptake. Kinetic studies showed a single saturable high-affinity carrier with a Michaelis constant (Km) of 163 ± 23 μM and a maximum transport velocity (Vmax) of 13,713 ± 803 pmol·mg protein⁻¹·min⁻¹. Glutamine uptake was markedly inhibited in the presence of L-alanine, L-asparagine, and L-serine. Li⁺ did not substitute for Na⁺. These data show that L-glutamine is predominantly taken up through system ASC. Glutamine deprivation resulted in the decrease of glutamine transport by a mechanism that decreased Vmax without affecting Km. The expression of the system ASC subtype ASCT2 decreased in the glutamine-deprived group, whereas glutamine deprivation did not induce changes in system ASC subtype ASCT1 mRNA expression. Adaptive increases in Na⁺-dependent glutamate, Na⁺-dependent 2-(methylamino)isobutyric acid, and Na⁺-independent leucine transport were observed under glutamine-deprived conditions, which were completely blocked by actinomycin D and cycloheximide. These mechanisms may allow cells to survive and even grow under nutrient-deprived conditions.

AMINO ACID TRANSPORT across the plasma membrane is essential for supplying cells with amino acids for cellular metabolism (27). Transport is mediated via membrane proteins called carriers or transporters, which are responsible for the translocation of amino acid substrates from one side of the membrane to the other. Malignant cells display uncontrolled rates of cellular proliferation and require an increased supply of precursor amino acids to support key biosynthetic pathways (23). As a result, these cells have very efficient transport systems and can transport amino acids across the plasma membrane faster than normal cells (23). Human hepatoma cells, for example, transport glutamine at a rate 10–20 times faster than normal hepatocytes (5).

Glutamine provides nitrogen for a number of important precursors for macromolecule synthesis including purines, pyrimidines, amino sugars, and some amino acids (11). In addition, it is an important fuel for cancer cells. Glutamine metabolism may also supply precursors for the synthesis of glutathione, which serves as a major store of cellular reducing equivalents (3). In cancer cells, cell-growth rates and the biosynthesis of DNA and protein correlate directly with glutamine concentration in culture media (32). A significant correlation has also been found between glutamine transport and its disappearance rates (9). These findings suggest that glutamine transport is rate limiting for glutamine utilization.

Neuroblastomas are childhood tumors that are derived from neural crest cells. They are biologically remarkable in that some neuroblastomas regress spontaneously without chemotherapy, and spontaneous and induced maturation is seen with significant frequency (29). In contrast, many other neuroblastoma tumors show invasive and progressive growth behavior. The prognosis for this tumor can be determined using genetic markers such as a short-arm deletion on chromosome 1p (7), increased ploidy (1), and amplification of the N-myc oncogene (25). Although many tumor markers have been investigated with respect to the biology of neuroblastomas, a definitive and consistent causal pattern for the diverse behavior and variable biology of these tumors is still unexplained. Hannuniemi et al. (16) showed that the maximal velocities of the saturable influx of leucine, lysine, and glycine were greater in cultured neuroblastoma cells than in glioma cells. The contribution of the amino acid transport system (system A) is greater in a neuroblastoma cell line with no N-myc amplification (8). Because of the potential role of glutamine as a mediator of tumor growth, glutamine transport across the plasma membrane may be rate limiting for subsequent metabolic events in neuroblastomas. However, glutamine uptake by neuroblastoma cells has not been previously investigated.

Several studies have shown the adaptive regulation of amino acid transport in cells subjected to amino acid deprivation.
starvation (4, 10, 22, 30). For example, glutamine deprivation causes a significant increase in glutamine and glutamate transport velocity (22, 30). In neuroblastoma cells, the removal of glutamine from the culture medium resulted in a 10-fold increase in the specific activity of the enzyme glutamine synthetase compared with the basal level, which suggests a high capacity for glutamine utilization (20). It remains unknown how neuroblastoma cells regulate amino acid transport activities when the availability of key nutrients such as glutamine is limited.

The purpose of this study was to characterize glutamine transport across the plasma membrane of human neuroblastoma cells. In addition, we examined the effects of glutamine deprivation on the transport of glutamine and other amino acids. We used an SK-N-SH human neuroblastoma cell line because it provided a well-characterized in vitro model system in which to study the growth mechanism.

METHODS

Materials. The radiolabeled amino acids L-[3H]glutamine, L-[3H]glutamate, and L-[3H]leucine and the system A-specific substrate 2-[3H](methylamino)isobutyric acid (MeAIB) were obtained from Amersham (Arlington Heights, IL). Cell culture media were from GIBCO-BRL (Gaithersburg, MD). Amino acids and all biochemicals were purchased from Sigma Chemical (St. Louis, MO) and fetal bovine serum (FBS) was from JRH Biosciences (Lenexa, KS). Tissue-culture plates were obtained from Corning (New York, NY). Neuroblastoma cell line SK-N-SH was provided by Dr. Tadao Ohno (RIKEN Cell Bank, Tsukuba, Japan).

Cell culture. Neuroblastoma cells were cultured at 37°C in a humidified atmosphere of 5% CO2-95% air. Cells were maintained in DMEM supplemented with 2 mM L-glutamine, 10% FBS, 1,000 U/ml penicillin, and 1,000 U/ml streptomycin. The culture medium was changed every 3 days until cells were confluent. Cells were then used for experiments.

Amino acid transport measurement. Transport of L-[3H]glutamine was measured by the cluster-tray method of Gazzola et al. (13). Before use in transport assays, cells were rinsed twice with warm Na+-free Krebs-Ringer phosphate buffer (CholKRP, made by replacing corresponding Na+ salts with choline chloride and choline phosphate) to remove extracellular Na+ and amino acids. Radiolabeled glutamine (5 μCi of [3H]glutamine/ml) transport assays were performed for 1 min at 37°C with 10 μM unlabeled glutamine in both Na+-Krebs-Ringer phosphate (NaKRP) and CholKRP buffers. The transport reaction was terminated by discarding the uptake buffer and rinsing the cells three times with ice-cold CholKRP buffer (2 ml per well per rinse). The cells containing the cells were allowed to dry and were then solubilized in 200 μl of 0.2 N NaOH-0.2% SDS solution. The cell extract (100 μl) was neutralized with 10 μl of 2 N HCl and subjected to scintillation spectrometry. The remaining 100 μl in each well was used for a bicinchoninic acid protein assay (28). Na+-dependent glutamine transport values were obtained by subtracting the transport values in CholKRP from those in NaKRP. Saturable Na+-independent transport values were determined in CholKRP by subtracting the values in the presence of excess (10 mM) unlabeled glutamine from those in its absence. Transport of L-[3H]leucine, L-[3H]glutamate, and [3H]MeAIB was measured by the same method. Transport velocities were expressed in picomoles per milligram of protein per minute. Data (means ± SD) were analyzed and compared with Student’s t-test or one-way ANOVA. P < 0.05 was considered statistically significant.

RESULTS

Time course of [3H]glutamine transport. Na+-dependent glutamine uptake was found to be linear for at least 3 min, and the Na+-dependent component was shown to account for >95% of the total glutamine uptake at all time points. Therefore, 1 min was chosen for the measurement of the initial rate of Na+-dependent glutamine transport for subsequent experiments.

Kinetics of [3H]glutamine transport. Na+-dependent glutamine uptake was determined at glutamine concentrations between 10 μM and 1 mM in both Na+ and choline buffers. A saturation plot of uptake velocity as a function of glutamine concentration is shown in Fig. 1A. When an Eadie-Hofstee plot was created for each kinetic study, the data were found to fit a single linear regression line. A representative plot is shown in Fig. 1B.
1B. When the data from three separate kinetics studies were averaged, they demonstrated a single high-affinity transport carrier for glutamine with a Michaelis constant \((K_m)\) of 163 ± 23 μM and a maximum transport velocity \((V_{\text{max}})\) of 13,713 ± 803 pmol·mg protein\(^{-1}\)·min\(^{-1}\).

Amino acid inhibition of \(^{3}H\)glutamine transport. To characterize the Na\(^+\)-dependent glutamine transport system, glutamine transport was measured in the presence of 5 mM concentrations of selected amino acids. The osmotic effects of inhibitors were compensated for by the addition of 5 mM sucrose to control assays. The data are expressed as a percentage of the control rate of glutamine uptake (Fig. 2). Na\(^+\)-dependent glutamine transport was significantly inhibited by L-glutamate, L-alanine, L-serine, and L-asparagine (substrates for system A; P < 0.001) but was unaffected by L-glutamate and L-arginine. Neither MeAIB, a highly specific substrate for system A, nor 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid, a specific substrate for the Na\(^+\)-independent system L, demonstrated significant inhibition of glutamine uptake.

Effects of external pH on \(^{3}H\)glutamine transport. Na\(^+\)-dependent glutamine transport was measured at several pH values between 6.0 and 8.0. The pH of the extracellular medium was adjusted by adding varying fractions of bicarbonate. Chloride salts of Na\(^+\), Li\(^+\), or choline were added at 119 mM. Results represent Na\(^+\)-, Li\(^+\)-, or choline-dependent velocities only.

Effects of cations on \(^{3}H\)glutamine transport. To examine the ability of the transporter to exchange Li\(^+\) for Na\(^+\), Na\(^+\)-dependent glutamine transport was measured in the presence of 119 mM NaCl, LiCl, and choline chloride (Table 1). Na\(^+\)-dependent glutamine transport significantly decreased in the presence of Li\(^+\) and choline compared with Na\(^+\) (P < 0.001).

Effects of glutamine concentration on cell growth. The effect of glutamine concentration on cell growth was determined. Cells were seeded at a density of 1 × 10^5 cells/ml (1 ml/well) into 12-well tissue-culture plates. After 24 h, the culture medium was removed and changed to glutamine-free DMEM supplemented with 10% FBS plus various concentrations of glutamine (2 mM and 400, 200, 100, and 0 μM). Cells were detached from the plate with trypsin and counted at days 0, 1, 2, and 3 with a hemocytometer. Cell growth in 2 mM glutamine was chosen as the control. As shown in Fig. 3, cell-growth rates were dependent on glutamine concentrations. In 200 μM glutamine, cells grew about half as fast as controls. Cells showed slow growth even in 0 μM glutamine, and this glutamine concentration was used for subsequent experiments as the glutamine-deprived condition.

Time-dependent effects of glutamine deprivation on \(^{3}H\)glutamine transport. Cells were seeded into 24-well tissue-culture plates (0.5 ml/well). At 100% cell confluence, the culture medium was removed and changed to glutamine-free DMEM supplemented with 10% FBS plus 2 (control) or 0 (glutamine deprivation) mM glutamine. Na\(^+\)-dependent glutamine transport was measured at 0, 8, 16, and 24 h. As shown in Fig. 4A, Na\(^+\)-dependent glutamine transport in the glutamine-deprived cells decreased significantly compared with control at 8 h (control, 1,320 ± 14; glutamine deprivation, 1,159 ± 186 pmol·mg protein\(^{-1}\)·min\(^{-1}\); P < 0.05), 16 h (control, 1,638 ± 26; glutamine deprivation, 1,185 ± 77 pmol·mg protein\(^{-1}\)·min\(^{-1}\); P < 0.01), and 24 h (control, 1,424 ± 33; glutamine deprivation, 983 ± 154 pmol·mg protein\(^{-1}\)·min\(^{-1}\); P < 0.01). To determine the kinetics of the glutamine-deprived effects on glutamine transport, the transport of

Table 1. Effect of substituting Li\(^+\) or choline for Na\(^+\) on Na\(^+\)-dependent glutamine transport in an SK-N-SH neuroblastoma cell line

<table>
<thead>
<tr>
<th>Cation</th>
<th>Na(^+)-Dependent Glutamine Transport, pmol·mg protein(^{-1})·min(^{-1})</th>
</tr>
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<tbody>
<tr>
<td>Na(^+)</td>
<td>1,324 ± 169</td>
</tr>
<tr>
<td>Li(^+)</td>
<td>24 ± 1*</td>
</tr>
<tr>
<td>Choline</td>
<td>98 ± 15*</td>
</tr>
</tbody>
</table>

Values are means ± SD of quadruplicate determinations. Glutamine transport at 10 μM was measured for 60 s. All assays were buffered with Krebs-Ringer solutions containing 25 mM choline bicarbonate. Chloride salts of Na\(^+\), Li\(^+\), or choline were added at 119 mM.
glutamine from 10 μM to 1 mM was determined in both control and glutamine-deprived cells (Fig. 4B). There was a significant decrease in maximum transport velocity in the glutamine-deprived group compared with control (V_max: control, 13,713 ± 803; glutamine deprivation, 9,553 ± 646 pmol·mg protein⁻¹·min⁻¹; P < 0.01), but no significant change was observed in transport affinity (K_m: control, 163 ± 23; glutamine deprivation, 181 ± 18 μM).

Effects of glutamine deprivation on expression of system ASC subtypes. The effect of glutamine deprivation on the expression of different subtypes of system ASC (ASCT1 and ASCT2) was analyzed using RT-PCR (Fig. 5). Cells were seeded at a density of 1 × 10⁵ cells (2 ml/well) into six-well tissue-culture plates. After 100% cell confluence was attained, the culture medium was removed and replaced with glutamine-free DMEM supplemented with 10% FBS plus 2 (control) or 0 (glutamine deprivation) mM glutamine. At 0, 12, and 24 h after the medium was changed, total RNA was extracted from the cells using a RNase Total Pure Purifications kit (Bioline, London, UK). The amount of extracted RNA was calculated from optical density measurements at 260 nm. RNA (1.0 μg) was used to generate first-strand complementary DNA using an Advantage RT for PCR kit (Clontech, Palo Alto, CA) according to the manufacturer’s recommended procedures. RT-PCR was performed using the same complementary DNA samples with primer pairs specific for human ASCT1, ASCT2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using a Takara PCR Thermal Cycler. The primer pairs used were (forward) 5’-AGT ATC AGA GGC AGG TCA TGA-3’ for ASCT1; (forward) 5’-CAA GGA GGT GCT CGA TTC GT-3’, (reverse) 5’-ACC CTG GTT CCG GTG ATA TTC-3’ for ASCT2; and (forward) 5’-GAA GGT GAA GGT CGG-3’, (reverse) 5’-GAA GAT GGT GAT GGG-3’ for GAPDH. Amplification was initiated by a 10-min denaturation at 95°C followed by 40 cycles at 95°C for 15 s and 60°C for 60 s. After the last cycle, the samples were incubated for 10 min at 72°C. The PCR products were then visualized by ultraviolet illumination after electrophoresis through 1.6% agarose gels containing 0.5 μg/ml ethidium bromide. The gel photographs were scanned with a computerized densitometer. Semiquan-

Fig. 3. Effects of glutamine deprivation on cell growth in an SK-N-SH neuroblastoma cell line. Cell numbers were counted on days 0, 1, 2, and 3 after changing the culture medium to glutamine-free DMEM supplemented with 10% fetal bovine serum (FBS) plus various concentrations of glutamine (2 mM and 400, 200, 100, and 0 μM). Estimated glutamine concentration of 10% FBS is 60 μM. Data are presented as the mean cell number ± SD of triplicate determinations. Where not shown, error bars are within the symbols.

Fig. 4. A: time-dependent effects of glutamine deprivation on Na⁺-dependent glutamine transport in an SK-N-SH neuroblastoma cell line. Glutamine (10 μM) transport was assayed for 60 s at 0, 8, 16, and 24 h after culture medium was changed to glutamine-free DMEM supplemented with 10% FBS plus 2 (control) or 0 (glutamine deprivation) mM glutamine. Estimated glutamine concentration of 10% FBS is 60 μM. B: Eadie-Hofstee plot of saturable Na⁺-dependent glutamine transport in a control and glutamine-deprived human neuroblastoma cell line. Transport velocity is plotted against velocity/[glutamine]. Data are presented as means ± SD of triplicate determinations. Where not shown, error bars are within symbols; *P < 0.05, **P < 0.01 vs. control.
and [3H]MeAIB was measured using the same method as described for glutamine transport measurement. The transport of these amino acids was linear for at least 3 min, and Na+-dependent glutamate (system XAg), Na+-dependent MeAIB (system A), and Na+-independent leucine (system L) uptake represented at least 70%, 60%, and 95% of the total uptake, respectively. Therefore, 1-min assays of Na+-dependent glutamate, Na+-dependent MeAIB, and Na+-independent leucine transport were chosen for subsequent experiments.

As shown in Fig. 6, there was a significant increase in the transport velocities of Na+-dependent glutamate (32 ± 6 pmol·mg protein⁻¹·min⁻¹; P < 0.05), Na+-dependent MeAIB (72 ± 2 pmol·mg protein⁻¹·min⁻¹; P < 0.001), and Na+-independent leucine (2,997 ± 333 pmol·mg protein⁻¹·min⁻¹; P < 0.001) in the glutamine-deprived cells compared with control (glutamate, 22 ± 2; MeAIB, 46 ± 6; and leucine, 1,549 ± 78 pmol·mg protein⁻¹·min⁻¹). Actinomycin D and cycloheximide completely blocked the glutamine deprivation-induced increase in transport of these amino acids, and transport activity was returned to the basal levels found in the control. In the glutamine-deprived group, actinomycin D and cycloheximide further decreased the Na+-dependent glutamine-transport activity that had been attenuated by glutamine deprivation. Na+-dependent glutamate (1,488 ± 59 pmol·mg protein⁻¹·min⁻¹) and Na+-dependent MeAIB transport in the control group significantly decreased with actinomycin D treatment (glutamine, 843 ± 88 pmol·mg protein⁻¹·min⁻¹; P < 0.001; MeAIB, 35 ± 6 pmol·mg protein⁻¹·min⁻¹; P < 0.05) and with cycloheximide treatment (glutamine, 899 ± 45 pmol·mg protein⁻¹·min⁻¹; P < 0.001; MeAIB, 20 ± 4 pmol·mg protein⁻¹·min⁻¹; P < 0.001), but neither had an effect on Na+-dependent glutamate and Na+-independent leucine transport.

DISCUSSION

Our study is the first to characterize the details of the glutamine transport system in a human neuroblastoma cell line. Ninety-five percent of glutamine transport by SK-N-SH cells occurred via carrier-mediated Na+-dependent transport pathways. Kinetic analysis demonstrated a single high-affinity transport carrier for glutamine with a K_m of 163 μM. This transporter has high affinity, because the circulating concentrations of glutamine are 600–700 μM or about four times greater than the K_m. Thus even when circulating concentrations of glutamine are very low, such as in a poorly vascularized portion of a solid tumor, the glutamine carrier should still be saturated. This ability to utilize glutamine may be essential to support proliferation, energy metabolism, and protein synthesis.

In the past, a large number of mammalian amino acid transport systems were studied using membrane vesicle preparations or cultured cells (6, 12, 17, 19). These included groups of Na+-dependent amino acid transporters that utilize free energy stored as a Na+ electrochemical potential gradient across plasma membranes for the uphill transport of amino acids. The Na+-dependent transporters play a central role in amino acid mobilization in animals because of their ability to transport amino acids against a concentration gradient. Criteria used to differentiate amino acid transport systems in mammalian cells to include substrate specificity, analog cross-inhibition patterns, transport kinetics, and ion dependency (27). Because MeAIB did not interfere with glutamine transport, system A is not an important transport system for...
glutamine. Another Na\textsuperscript{+}-dependent transporter, which is named system N for the nitrogen-containing side chain of its substrates, is characterized by histidine transport inhibition of glutamine (19). In our studies, histidine only partially blocked glutamine transport, and Li\textsuperscript{+} did not substitute for Na\textsuperscript{+} as a cation (as it does for system N). The inability of Li\textsuperscript{+} to support glutamine transport in conjunction with the inhibitor profile indicates that system N is not a major component of glutamine transport. In summary, based on the inability of this high-affinity carrier to transport glutamine in the presence of alanine, serine, or asparagine, its intolerance to N-methylated substrate, and the failure of Li\textsuperscript{+} to substitute for Na\textsuperscript{+}, we conclude that glutamine is predominantly taken up through system ASC in SK-N-SH cells.

The relative pH insensitivity of system ASC has been a useful parameter in differentiating it from systems A and N (27). However, in our studies, glutamine transport decreased at pH 6.0 and 6.5 compared with pH 7.5. Handlogten et al. (15) obtained similar results and reported that system ASC was inhibited when the pH was lowered from 6.5 to 5 in a hepatoma cell line. In the brush-border membrane vesicles of rabbit ileum, system ASC increased at lower pH (24). Therefore, the pH sensitivity of system ASC is not specific to the SK-N-SH neuroblastoma cell line. Our finding suggests that pH changes may serve as important regulatory steps in subsequent intracellular metabolism in SK-N-SH cells.

Glutamine deprivation resulted in the decrease of glutamine transport by a mechanism that decreased V\textsubscript{max} without affecting K\textsubscript{m} (see Fig. 4). This indicates that the number of active glutamine transporters in the cell membrane decreased without affecting the affinity of the transporter. An increase in the number of active amino acid transporter proteins in the cell membrane usually involves an increase in de novo protein synthesis of the carrier itself (27). Therefore, the decrease in glutamine transporters may occur secondary to a decrease in the rate of de novo carrier biosynthesis or an increase in the rate of carrier breakdown. In contrast to glutamine transport, SK-N-SH cells responded to glutamine deprivation by increasing the transport activities of leucine, glutamate, and MeAIB. Na\textsuperscript{+}-independent leucine, Na\textsuperscript{+}-dependent glutamate, and Na\textsuperscript{+}-dependent MeAIB are transported via systems L, X\textsubscript{AG}, and A, respectively (19). The adaptive increases in amino acid transport elicited by glutamine deprivation have already been shown in human hepatoma cell lines (33). Kilberg et al. (18) showed that system A increased after amino acid deprivation in rat H4 hepatoma cells, whereas other transport systems such as ASC, N, y\textsuperscript{+}, and L were unaffected. It is possible that differences in cellular metabolism underlie the disparity in the transporter response to glu-
tamine deprivation among the cell lines. Because glutamine is used in several disparate metabolic pathways, SK-N-SH neuroblastoma cells may replace metabolic intermediates normally provided by glutamine with the carbon skeletons and nitrogen of other amino acids when extracellular glutamine levels are diminished.

The first mammalian glutamine transporter gene was isolated in 1996 from a mouse testis cDNA library; it encoded a 553-amino acid protein with functional properties of system ASC (31). It was called ASC2 to distinguish it from ASCT1, a system ASC isoform isolated in 1993 that does not transport glutamine (2, 26). ASC2, which exhibits 57% sequence identity to ASCT1, takes up glutamine with high affinity and transports a wide panel of other amino acids including serine, threonine, cysteine, alanine, and asparagine as well as branched-chain amino acids (leucine, valine, and isoleucine) to a lesser degree (31). There have been no studies of the molecular regulation of system ASC transporter genes in amino acid-deprived conditions. In this study, ASC2 mRNA expression decreased in the glutamine-deprived group, whereas glutamine deprivation did not induce changes in ASCT1 mRNA expression, which shows that the decrease in ASC2 mRNA expression is comparable to that observed for the glutamine transport activity in the glutamine-deprived group. Therefore, we conclude that glutamine is taken up via a system ASC subtype, ASC2, and down-regulation of glutamine transport induced by glutamine deprivation is due to modifications in the expression of ASC2-related genes.

The increased amino acid transport that was induced by glutamine deprivation was completely blocked by the RNA synthesis inhibitor actinomycin D and by the protein synthesis inhibitor cycloheximide. In our unpublished data, leucine transport increased in the glutamine-deprived group by a mechanism that increased V_{max}. Amino acid starvation increased the expression of the ATA2 system A transporter gene in human hepatocytes (34). Based on these data, one possible explanation for the different responses to the inhibitors is that the half-life of the transporter proteins of systems A and ASC are shorter than those of systems L and X_{AG}.

As shown in Fig. 3, the growth of SK-N-SH cells was less sensitive to glutamine deprivation than has been reported for other cancer cell lines (32). The SK-N-SH cells survived and even grew in glutamine concentrations lower than normal circulating levels (500–600 μM). The centers of solid tumors are generally poorly vascularized, and intracellular amino acid concentrations can be much lower than the normal circulating levels (27). Decreased extracellular amino acid levels encountered by tumors in vivo may elicit similar adaptive responses in amino acid transport that contribute to the maintenance of cytoplasmic amino acid levels that are essential for growth.

REFERENCES


