The A, ASC, and L Systems for the Transport of Amino Acids in Chinese Hamster Ovary Cells (CHO-K1)*

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The neutral amino acid transport systems in Chinese hamster ovary cells (CHO-K1) have been characterized by examining primarily the transport of alanine and tryptophan. Tryptophan is transported almost exclusively through the L system (Km = 0.15 mM, Vmax = 2.1 mm/min). This system is Na+-independent, is not inhibited by α-(methylamino)isobutyric acid (MeAIB), is inhibited exclusively by typical L system amino acids, transports these amino acids at high velocities, exhibits accelerative exchange diffusion, and is trans-inhibited by alanine.

Eleven percent or less of the initial velocity of alanine uptake occurs by way of the Na+-independent L system. The A system, defined primarily by its sensitivity to inhibition by MeAIB and its Na+-dependent and ability to substitute Li+ for Na+, is the minor system for alanine transport (Km = 0.82 mM, Vmax = 0.72 mm/min) while the ASC system (Km = 0.12 mM, Vmax = 0.84 mm/min) is the major one. Proline and α-aminoisobutyric acid (AIB) are the only amino acids found that are transported to a larger extent (but still at a comparatively low rate) by the A as compared to the L or ASC systems. AIB is not an A system-specific amino acid. In these cells, the ASC system is not inhibited by MeAIB, is Na+-dependent, cannot substitute Li+ for Na+, and is the major system for the transport of the small chain amino acids. Serine is the preferred substrate and appears to be relatively specific for this system (Km = 0.52 mM, Vmax = 2.5 mm/min). This system also transports isoleucine, methionine, valine, and phenylalanine at significant rates. 2-Aminobicyclo(2,2,1)heptane-2-carboxylic acid inhibits alanine transport through the ASC system. The possible presence of another minor alanine transporting system inhabitable by MeAIB is discussed.

In 1963, Oxender and Christensen (1) defined two distinct transport systems with overlapping specificities for the uptake of the neutral amino acids in Ehrlich ascites cells. System A was shown to handle mainly amino acids such as alanine, serine, glycine, and the amino acid analogue α-aminoisobutyric acid while System L was shown to have a preference for the branched chain and aromatic amino acids. Some amino acids, such as methionine and amino acids with long straight hydrocarbon side chains, were transported equally well by both systems. In addition to these differences in amino acid specificity, the two systems also differed with regard to dependence on Na+ ion, pH optimum, sensitivity to metabolic inhibitors, trans-stimulation, and concentrative capacity.

Subsequently, a more detailed analysis of these systems in Ehrlich ascites cells revealed the presence of an additional transport system, the ASC system (2, 3), which showed a strong preference for alanine, serine, and cysteine. Systems similar to the A, ASC, or L systems have been characterized in a number of different cell lines (4-16).

Our laboratory has been employing a mutational approach for the elucidation of the mechanism(s) of amino acid transport in a number of different mammalian cell lines and for determination of the possible connection between the various systems involved (5, 6, 11, 13, 14). We have recently expanded this study to include the Chinese hamster ovary cell line CHO-K1 (17) because it is easy to culture and is widely used in many genetic and biochemical studies. To pursue this approach, we have characterized and reported here the systems for neutral amino transport in this cell line. Oxender et al. (10) briefly defined but did not fully characterize an A and L system in a cell line similar to CHO-K1. The possible presence of an ASC system was not explored so that their characterization of the A system has been complicated by the activity of an ASC system.

MATERIALS AND METHODS

Cells, Media, and Culture Conditions—The Chinese hamster ovary cell line CHO-K1 (17) obtained from the American Type Culture Collection (CCL 61) was used throughout these studies. Cells were routinely grown in Eagle's minimum essential medium (Flow Laboratories AutoPow MEM) with 10% or 7.5% fetal calf serum (Flow), 1 mM sodium pyruvate (Flow), 0.1 mM nonessential amino acids (Flow), 100 IU/ml of penicillin G (Eli Lilly and Co.), and 100 μg/ml of streptomycin (Eli Lilly and Co.). This medium is referred to as MEMCHO. Cultures were incubated at 37 °C in a humid atmosphere containing 5% CO2 and 95% air.

Chemicals—The amino acids (all L-isomers) were supplied by Calbiochem. The amino acid analogue, MeAIB* was supplied by Sigma. The analogue, BCH was initially a generous gift from H. N. Christensen (University of Michigan, Ann Arbor, MI) and was later purchased from Calbiochem. Tritiated compounds were purchased from either Amersham or ICRN Radiochemicals. Crystalline bovine serum albumin and cycloheximide were acquired from Calbiochem. NCS Tissue Solubilizer was supplied by Amersham.

Cell Maintenance—Cells were maintained as previously described (5). New cultures were initiated from frozen ampules approximately every 6 weeks. Cells were grown to be mycoplasma-free by the uridine, uracil methods of Schneider et al. (18).

Determination of Cell Size—Cell diameter and volume were determined using a Coulter Counter (Model Z) previously calibrated using ragweed pollen as a standard. The volume of CHO-K1 was determined to be 1.48 × 10−13 liters/cell.

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The abbreviations used are: MeAIB, α-(methylamino)isobutyric acid; AIB, α-aminoisobutyric acid; BCH, b-(±)-2-aminobicyclo-(2,2,1)heptane-2-carboxylic acid.
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Transport Studies—We performed uptake studies with cells grown in a monolayer at the bottom of scintillation vials by a modification of a procedure previously described (5). Exponential phase cells were inoculated into sterile scintillation vials at 10⁶ cells/vial. The cells were incubated for 24-36 h at 37 °C. At this time, the cells in 3 vials were removed and counted (usually 2.0-2.5 × 10⁶ cells/vial). For uptake, cells were washed 3 times with 3 ml of PBSMgA. Trytophan uptake at the concentration of 6.5 mM was determined by the absence of Na⁺ (Fig. 1A). Amino acid transport by the L system (in Ehrlich ascites cells) appear not to be dependent upon exchange diffusion (1, 3, 12, 13). In contrast, uptake of tryptophan was linear for just 5 s at concentrations from 0.01 mM to 0.3 mM. The zero time counts were averaged and subtracted from the averaged time interval counts when exit of the externally given substrate is insignificant.

RESULTS

Kinetics of Alanine and Tryptophan Uptake in the Presence of Na⁺—We measured the initial velocities of alanine and tryptophan uptake at 15-s intervals for the first 1 min and then at 2.5, and 10 min. We found that the uptake of alanine, at the concentrations employed (0.002 mM to 5 mM), was linear for 1 min. In contrast, uptake of tryptophan was linear for just 30 s at concentrations from 0.01 mM to 0.3 mM.

On the basis of these time course experiments, we determined the Michaelis-Menten parameters for alanine and tryptophan transport by measuring the initial velocities of uptake, in duplicate or triplicate as indicated, at a 1-min and at a 15-s time interval, respectively. Velocities were corrected for nonsaturable uptake (19) and were then graphed as a Line-Weaver-Burk plot. The plot of alanine uptake deviates slightly from linearity. As a first approximation, we fitted the alanine data to a single straight line and determined the kinetic parameters using a linear least squares and jackknife program (20, 21) (see Table I). The plot of tryptophan uptake is linear suggesting a single system for the transport of this amino acid (Fig. 1). The kinetics parameters were determined as described above and are listed in Table I.

The Effect of Na⁺ on Transport—It has been previously shown that transport through both the A and ASC systems is Na⁺-dependent, while transport through the L system is relative Na⁺-independent (1-4, 12, 14-16). We tested the sodium requirement for the transport of 0.3 mM alanine and 0.3 mM tryptophan (Fig. 2). Under conditions of no Na⁺, choline chloride at equal molarity was used in place of sodium chloride in PBSMgA. In the absence of Na⁺, the rate of alanine transported was only 11% of that in the presence of Na⁺. Because of this small uptake, we were unable to determine the kinetic parameters for alanine transport with sufficient accuracy under these conditions. We did find that the per cent of Na⁺-independent transport of alanine (at concentrations up to at least 0.5 mM) was 11% or less. Uptake of tryptophan, on the other hand, was not significantly altered by the absence of Na⁺ (Fig. 2). We have obtained similar results with phenylalanine (data not given).

Effect of Preloading on Transport—Transport by the L system using cells undepleted of the soluble amino acid pool probably occurs by exchange diffusion and the phenomenon of trans-stimulation observed has been explained on the basis of countercurrent or accelerating exchange diffusion (1, 3, 10, 21). In comparison, the A and the ASC systems (in Ehrlich ascites cells) appear not to be dependent upon exchange diffusion for activity and exhibit what seems to be weak accelerative exchange diffusion (1, 3). The phenomenon of accelerative exchange diffusion has been explained on the basis that the loaded carrier traverses the cell membrane from inside to outside and vice versa at a faster rate than the unloaded carrier. This model is distinguishable from another form of trans-stimulation that is the result of competition for exit, i.e. isotopic dilution or competitive exchange diffusion (22, 23). In many cases, to decide between the two alternative mechanisms, it is necessary (for instance, when measuring entry) to determine the influxes at the smallest possible time intervals when exit of the externally given substrate is insignificant.

In preliminary experiments, we determined that cells preincubated with 5 mM tryptophan (for 20 min in the presence of Na⁺) apparently accumulated 6.5 mM tryptophan and this concentration was saturating for maximum trans-stimulation when the external tryptophan concentration was over the Kₑ for tryptophan influx.

On the basis of these preliminary results, we preloaded the cells with 6.5 mM tryptophan (internal concentration) and compared the rates of tryptophan uptake at a concentration of 0.3 mM. The results (Fig. 3A) demonstrate that the rate of tryptophan uptake in cells that were not preloaded (starved cells) is linear for 30 s, and, at this point, the rate of tryptophan uptake is twice the rate of uptake in the cells that were preloaded (starved cells).

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The initial velocities of tryptophan uptake were determined for 15 s, in duplicate, at concentrations from 0.002 to 0.3 mM as described in Fig. 1. In Experiment 1, the initial velocities of alanine uptake were determined for 1 min, in triplicate, at concentrations from 0.005 to 5 mM. In Experiment 2, the initial velocities of alanine uptake were determined for 1 min, in triplicate, in the presence and absence of 10 mM MeAIB at concentrations of alanine from 0.01 to 0.5 mM as described in Fig. 6. The kinetic constants for alanine transport through the A system were derived from the differences in uptake velocities determined in the presence and absence of MeAIB. The kinetic constants for alanine transport through the ASC system were derived from the velocities determined in the presence of MeAIB.

### Table I. Kinetic parameters for the transport of tryptophan, alanine, MeAIB, and serine by CHO-K1

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>System</th>
<th>( V_{max} ) (mmol/min)</th>
<th>( K_m ) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>L system</td>
<td>2.1 (2.3-2.0)</td>
<td>0.013 (0.016-0.011)</td>
</tr>
<tr>
<td>Ala</td>
<td>Experiment 1</td>
<td>1.2 (1.4-1.2)</td>
<td>0.17 (0.20-0.14)</td>
</tr>
<tr>
<td>Ala</td>
<td>Experiment 2</td>
<td>1.3 (1.5-1.2)</td>
<td>0.17 (0.21-0.14)</td>
</tr>
<tr>
<td>Ala</td>
<td>Combined A and ASC</td>
<td>0.72 (1.0-0.50)</td>
<td>0.82 (1.4-0.48)</td>
</tr>
<tr>
<td>Ala</td>
<td>A system</td>
<td>0.84 (0.88-0.80)</td>
<td>0.12 (0.15-0.10)</td>
</tr>
<tr>
<td>Ala</td>
<td>ASC system</td>
<td>0.62 (0.77-0.49)</td>
<td>2.2 (3.0-1.6)</td>
</tr>
<tr>
<td>Serine</td>
<td>Combined A and ASC</td>
<td>2.5 (2.9-2.2)</td>
<td>0.22 (0.26-0.19)</td>
</tr>
<tr>
<td>MeAIB</td>
<td>Combined A and ASC</td>
<td>1.2 (1.4-1.2)</td>
<td>0.17 (0.20-0.14)</td>
</tr>
</tbody>
</table>

![Fig. 1. Kinetics of tryptophan transport in CHO-K1. Initial rates of uptake (15 s) were measured for concentrations of tryptophan ranging from 0.002 to 0.3 mM, in duplicate, as described under "Materials and Methods." The velocities were averaged and converted to millimolar concentrations min” (mmol/liter/min), and analyzed by a linear least squares fitting and jackknife error analysis computer program as described under "Materials and Methods." The numbers in parentheses are the upper and lower confidence limits as determined by the jackknife error analysis procedure (20).](image1)

![Fig. 2. The effect of Na⁺ on transport in CHO-K1. Uptake of 0.3 mM tryptophan and 0.3 mM alanine were measured, in triplicate, in the presence and absence of Na⁺ as described under "Materials and Methods." For conditions of no Na⁺, choline chloride (0.14 M) replaced the NaCl in the uptake buffer (PBSOMGA). Alanine, with Na⁺; C; alanine, no Na⁺; O, tryptophan, with Na⁺; △, tryptophan, no Na⁺. ▲.](image2)

We also tested for the trans-stimulation of alanine uptake. We preincubated cells with 5 mM alanine for 20 min and measured the initial rate of uptake of 3 mM alanine, a concentration 4 and 25 times the \( K_m \) for the low affinity (A) and high affinity (ASC) systems for alanine uptake, respectively, plus 0.14 mM tryptophan to exclude alanine from going in by the L system. We found that the rate of alanine uptake was linear for at least 1 min (Fig. 3b). At 15 and 30 s, there is a negligible effect of preloading on the rate of alanine uptake. At 2 min, there is a 1.2-fold increase in the preloaded cells, and at 5 min, when uptake of alanine in the starved cells deviates from linearity, there is a 1.6-fold increase. These results leave no doubt that accelerative exchange diffusion is not a significant

uptake in the preloaded cells is 2.5-fold greater than that obtained in starved cells. This increase in rate of tryptophan uptake at the linear portion of the curve excludes competitive exchange diffusion as an explanation of this phenomenon and supports the hypothesis of accelerative exchange diffusion or countertransport. However, the large differences in the uptake of tryptophan observed after 30 s, where the uptake of tryptophan in cells that were not preloaded deviates sharply from linearity, is no doubt due to the added effect of competitive exchange diffusion.

We also measured trans-stimulation in the reverse direction in which the exit of labeled tryptophan from preloaded cells was measured in the presence and absence of external tryptophan. This method has the advantage of eliminating recycling of the label back into the cells because of the large dilution. The results of this experiment show approximately a 2-fold stimulation of exit as a result of the presence of external tryptophan (Table II).

To examine the specificity of this event, we tested the effect of preloading the cell with different concentrations of alanine on the influx of tryptophan in the presence of Na⁺. Alanine not only failed to stimulate but actually inhibited tryptophan uptake by 67% (Table III).
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**Fig. 3.** The effect of amino acid preloading on the transport of tryptophan and alanine in CHO-K1. a, the uptake of 0.3 mM tryptophan was measured in duplicate, in cells starved for amino acids (●) and in cells preincubated with 5 mM tryptophan (○) as described under "Materials and Methods." b, the uptake of 3 mM alanine was measured in the presence of 0.14 mM tryptophan, in duplicate, in cells starved for alanine (●) and in cells preincubated with 5 mM alanine (○) as described under "Materials and Methods."

**TABLE II**

### Exit of tryptophan from preloaded cells

<table>
<thead>
<tr>
<th>External tryptophan concentration (mM)</th>
<th>Amount of tryptophan exiting in 1 min (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.06</td>
</tr>
<tr>
<td>0.01</td>
<td>0.74</td>
</tr>
<tr>
<td>0.1</td>
<td>0.91</td>
</tr>
<tr>
<td>1.0</td>
<td>1.08</td>
</tr>
<tr>
<td>5.0</td>
<td>1.14</td>
</tr>
</tbody>
</table>

**TABLE III**

### Effect of preloaded alanine on tryptophan uptake

<table>
<thead>
<tr>
<th>Internal alanine concentration (mM)</th>
<th>One minute uptake of 0.018 mM tryptophan (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.30</td>
</tr>
<tr>
<td>0.06</td>
<td>0.24</td>
</tr>
<tr>
<td>0.6</td>
<td>0.25</td>
</tr>
<tr>
<td>5.2</td>
<td>0.15</td>
</tr>
<tr>
<td>8.3</td>
<td>0.14</td>
</tr>
<tr>
<td>16.7</td>
<td>0.11</td>
</tr>
<tr>
<td>17.9</td>
<td>0.11</td>
</tr>
</tbody>
</table>

"The internal, preloaded alanine concentration was determined, as described under "Materials and Methods," using increasing concentrations of alanine and [1H]alanine.

The amount of [1H]tryptophan transported into the cell in 1 min was determined in triplicate, as described under "Materials and Methods," using cells preloaded with various amounts of unlabeled alanine as indicated.

factor in alanine uptake. The relatively small increases in rates, as a result of preloading, at the longer time intervals (where isotope dilution would be expected) indicate that the major systems involved in alanine transport, A and ASC, are only weakly subject to exchange diffusion.

**Competition Studies**—We first measured the effect of 0.1 mM concentrations of a number of amino acids on the uptake of labeled 0.01 mM tryptophan and found that the amino acids fell into two classes (Fig. 4). One class that inhibited tryptophan uptake by 70–90% is represented by the long and branched chain and aromatic amino acids, amino acids which are mainly transported by the L system (at 0.1 mM, see below). A second class that is not inhibitory is represented by the small chain amino acids such as alanine, serine, threonine, and proline, amino acids transported mainly by the A or ASC system (at 0.1 mM, see below) and the positively charged amino acids. Similar results were obtained when the uptake of 0.3 mM labeled tryptophan was measured in the presence of 3 mM concentrations of these same amino acids (data not given).

Alanine uptake was measured at 0.01 mM alanine or 0.5 mM alanine ± 10 times the concentrations of a test amino acid. At 0.01 mM alanine, the results indicate that cysteine, threonine, and serine, amino acids that have been shown to be mainly transported by the ASC system in Ehrlich ascites cells (3) and in CHO-K1 (see below), were the best inhibitors reducing alanine uptake by 50–70%. On the other hand, glycine, proline, AIB, and MeAIB (MeAIB is specific for the A system in the Ehrlich ascites cells (3), 2, 3) were relatively ineffective as inhibitors. The long and branched chain and aromatic amino acids, and histidine were also poor inhibitors of alanine uptake (inhibition 0–15%).

When we tested the effect of competing amino acids (5 mM) on the transport of 0.5 mM alanine in the absence of Na⁺, conditions in which the L system would be the predominating system or the only functioning system, we found that the velocity of 0.5 mM alanine transport was reduced by 92% (by leaving out Na⁺) and that the branched and long chain and aromatic amino acids became very inhibitory. (Compare transport of alanine at 0.5 mM in the presence and absence of Na⁺.)

**Kinetics of Alanine Transport through the ASC and A Systems**—We have shown that about 90% of alanine transport is Na⁺-dependent and that the small amount of Na⁺-independent transport is mediated through the L system. In competition experiments we showed that MeAIB, the specific
substrate for the A system in Ehrlich ascites cells, is a poor inhibitor of alanine transport, while typical ASC substrates such as serine, threonine, and cysteine are very strong inhibitors. This suggested that the A system is probably not the dominant Na⁺-dependent alanine transporting system in CHO-K1 at 0.01-0.5 mM alanine. To examine this possibility further, we determined the effect of increasing concentrations of MeAIB on the initial rate of transport of 0.01 mM alanine on the basis that MeAIB may have a relatively high Ka for the A system and that we may have, therefore, missed its inhibitory effect in our previous experiment. The results of two such experiments are shown in Fig. 5. At 0.5 mM, MeAIB becomes saturating, producing a maximum inhibition of about 20%. In other experiments, MeAIB inhibition was found to vary and we calculated the mean inhibition to be 13%. We also found that AIB inhibits alanine transport similarly.

In our studies of alanine transport, we investigated the possible use of BCH, a specific inhibitor of the L system in Ehrlich ascites cells (23), to block the L system in CHO-K1 cells. We found that BCH was relatively nonspecific in our cell line (Fig. 5). In measuring the velocity of alanine uptake at 0.01 mM in the presence of 2 mM MeAIB, we never reached saturation as we increased the BCH concentration. At 11 mM BCH, the velocity of alanine transport was inhibited by 50%. At this concentration of alanine, the per cent of the Na⁺-independent transport was less than 11%. Therefore, BCH, besides presumably inhibiting alanine transport through the L system, can also block alanine transport through the Na⁺-dependent systems. Because of the relatively small amount of alanine transported by the A system, and the presence of 2 mM MeAIB, BCH must inhibit alanine transport through the ASC system in this cell line.

On the basis that the velocity of uptake excluded by the addition of MeAIB represents alanine transport through the A system, we determined the Michaelis-Menten parameters for transport through the A system by measuring the initial velocity of alanine uptake in the presence and absence of 10 mM MeAIB and subtracting the former velocities from the latter. We also determined the parameters for alanine transport through the ASC system by utilizing the velocities of alanine uptake in the presence of MeAIB at alanine concentrations of 0.01 mM to 0.5 mM, concentrations where there is a relatively insignificant amount of alanine transported through the L system. Lineweaver-Burk plots of the initial velocities thus obtained appear linear (Fig. 6) and the kinetic parameters are listed in Table I. It can be seen that the ASC system is the high affinity system for alanine transport and that it accounts for the major portion of alanine transport.

To obtain a direct measure of the kinetic parameters of the A system, we measured the initial rate of MeAIB uptake. A Lineweaver-Burk plot of the initial velocities of MeAIB uptake from 0.2 to 5.0 mM appears linear, and the computer fit gives a Ka and Vmax of 2.2 mM and 0.62 mm/min, respectively (see Table I).

We measured the specificity of the ASC system by inhibition analysis by measuring the initial velocity of transport of 0.01 mM alanine in the presence of 10 mM MeAIB and 1.0 mM BCH, conditions that preclude transport through the A and L systems, respectively. (BCH at 1.0 mM will probably interfere slightly with transport through the ASC system.) We added the test amino acids at 1.0 mM since in preliminary experiments we had shown that serine completely saturates for inhibition at that concentration. It will be noted that serine, cysteine, and threonine, typical ASC amino acids (2, 3), are strong inhibitors, while methionine, tryptophan, and isoleucine are moderate, and phenylalanine and proline are weak inhibitors (Fig. 7).

To obtain a direct measure of the specificity of the ASC, A, and L systems, we measured individually the transport of 11 amino acids at 0.1 mM in the presence and absence of 10 mM MeAIB and also in the absence of Na⁺. It can be seen (Fig. 8) that serine at 0.1 mM is transported at a high velocity by, and is relatively specific for, the ASC system. This system accounts for 94% of the total uptake of serine. After serine, the amino acids alanine, threonine, and cysteine at 0.1 mM are next most preferred by the ASC system accounting for 85%, 72%, and 55% of their transport. Isoleucine, methionine, phenylalanine, leucine, and tryptophan are taken up by the ASC system but are mainly transported by the L system. The A system transports all these amino acids at relatively low velocities. Proline and AIB are the only amino acids found that are transported to a larger extent by the A system.

**Kinetics of Serine Transport through the ASC System**—We determined the kinetic parameters of serine transport at a concentration range from 0.005 mM to 5.0 mM as described under "Materials and Methods." Serine uptake was linear for

![Fig. 5. Inhibition of alanine uptake by MeAIB, AIB, and BCH in CHO-K1. One-min velocities of uptake of 0.01 mM alanine were measured in the presence of increasing concentrations of MeAIB, AIB, or BCH in triplicate, as described under “Materials and Methods.” The data for the AIB are the results of two different experiments. The observed velocities were averaged and presented as per cent of the control uptake (uptake in the absence of MeAIB, AIB, or BCH). MeAIB: △ AIB; O, BCH; □. The effect of increasing concentrations of BCH was measured in the presence of 2 mM MeAIB.](image-url)

![Fig. 6. Kinetics of alanine transport through the A and ASC systems in CHO-K1. Alamine transport was measured in 1-min velocities in triplicate, in the presence and absence of 10 mM MeAIB (a concentration of MeAIB that saturates the A system) as described under “Materials and Methods.” The observed velocities of uptake were averaged and converted to millimolar concentrations of alanine taken up per min. Combined uptake, the velocity in the absence of MeAIB, △; ASC uptake, the velocity in the presence of MeAIB, O; A uptake, the difference between the velocity in the presence and absence of MeAIB.](image-url)
Alanine transport, at 0.01 mM, was measured in triplicate in the presence of 10 mM MeAIB and 1 mM BCH plus 1 mM concentrations of other amino acids as described under "Materials and Methods." The velocities were averaged and presented as percent inhibition of the control uptake (uptake in the presence of MeAIB and BCH).

FIG. 7. Specificity of the ASC system by inhibition analysis.

Alanine transport, at 0.01 mM, was measured in triplicate in the presence of 10 mM MeAIB and 1 mM BCH plus 1 mM concentrations of other amino acids as described under "Materials and Methods." The velocities were averaged and presented as percent inhibition of the control uptake (uptake in the presence of MeAIB and BCH).

FIG. 8. A comparison of the relative velocities of transport of amino acids through the ASC, A, and L systems in CHO-K1. Amino acid uptake, at 0.1 mM, was measured in triplicate under three conditions: 1) in the presence of 10 mM MeAIB, 2) in the absence of MeAIB, and 3) in the absence of Na⁺ (choline chloride replacing sodium chloride). The velocity transported through the A system was calculated by subtracting the velocity determined in the absence of MeAIB from that determined in the absence of Na⁺. The velocity transported through the ASC system was calculated by subtracting the velocity in the absence of Na⁺ from that determined in the presence of MeAIB. The velocity transported through the L system was the velocity measured in the absence of Na⁺. Velocities were not corrected for nonsaturable uptake. Standard deviations are indicated. The first bar for each amino acid represents transport through the ASC system, the second bar transport through the A system, and the third bar transport through the L system.

FIG. 9. The effect of substituting Li⁺ for Na⁺ on alanine transport in CHO-K1. One-min velocities of uptake of 0.1 mM alanine were measured in triplicate, as described under "Materials and Methods," in the presence of increasing concentrations of MeAIB under three conditions: 1) in the presence of Na⁺ (O), 2) in the absence of Na⁺ (V), and 3) with Li⁺ replacing Na⁺ on an equimolar basis (O). It can be seen that the velocity of alanine transport inhibited by MeAIB, that is, velocity through the A system, accounts for 18% of the total velocity, while the L system accounts for just 5% of the velocity and the ASC system 78%. Substituting Li⁺ for Na⁺ reduced the total velocity of alanine uptake by 89% demonstrating the Na⁺ dependence for the ASC system. It appears that Li⁺ is able to partially substitute for Na⁺ in the transport of alanine by the A system.

DISCUSSION

By studying various aspects of tryptophan transport, and the transport of other amino acids in the absence of Na⁺, we have characterized the L system for amino acid transport in CHO-K1 cells. Although this system in CHO-K1 is similar to the L system of other mammalian cells (2, 10, 14), there is a striking difference in specificity of transport as inferred from competition studies and by direct measure of amino acid transport in the absence of Na⁺. Long and branched chain amino acids do strongly inhibit tryptophan transport in CHO-K1, which is what one might expect from previously described L systems. However, we found that amino acids such as alanine, serine, threonine, proline, and glycine are not inhibitory at concentrations 10 times the concentration of tryptophan employed. This is in contrast to what has been found in other cell lines (1, 2, 14) where these amino acids significantly inhibit L system amino acid transport. The L system in CHO-K1 also differs from the high affinity tryptophan transport system in human red blood cells (25). The latter system is specific for tryptophan and phenylalanine. Other amino acids that we have shown are transported mainly by the L system as well as those that are transported mainly by the A and ASC systems are poor inhibitors.

Another feature of the L system in CHO-K1 which deserves emphasis is the transinhibition of tryptophan uptake observed by preloading cells with alanine. Jacquez (26) was first to observe a trans-inhibition effect of preloaded glycine on tryptophan uptake. The phenomenon of trans-inhibition has been
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reported in the human lymphoblast (14) with regard to phenylalanine transport. Calculations reveal that the trans-inhibition cannot be explained by a cis-inhibition caused by exited alanine because of the large dilution effect and the fact that alanine is a poor cis-inhibitor of transport (K_1 = 2.4 mM, data not given).

If trans-stimulation, i.e. accelerative exchange diffusion, is due to the loaded carrier transferring the membrane faster than the unloaded carrier (22, 23), it is reasonable to propose that the carrier loaded with alanine might inhibit the reorientation of the carrier to the outside. Whether this is accomplished by alanine binding to the same site as for transport or to a unique regulatory site for A and/or ASC amino acids on the L system carrier is under investigation.

Besides the Na⁺-independent L system, our experiments indicate that there are at least two Na⁺-dependent systems for the transport of the neutral amino acids, systems analogous to the ASC and A system described in other cell lines.

By measuring uptake of alanine in the presence or absence of Na⁺, we determined that, at 0.3 mM alanine, only 11% of the alanine transport is Na⁺-independent. The fact that the Na⁺-independent transport of alanine is inhibited by typical L system amino acid indicates that this portion of alanine transport is mediated by the L system.

Christensen et al. (2, 3) have shown that MeAIB is a specific substrate for the A system in Ehrlich ascites cells. We found that MeAIB, when added in increasing amounts, was able to block and saturate a definite fraction of alanine transport, thus separating Na⁺-dependent transport into one component inhibited by MeAIB and another which was immune to this inhibitor. We estimate a K_1 for MeAIB inhibition of alanine transport at 0.2 mM. However, in measuring MeAIB transport kinetics between 0.2 and 5.0 mM, we determined a K_1 for MeAIB of 2.2 mM. (We were unable to employ lower MeAIB concentrations because of the poor uptake in relationship to background counts.) This apparent discrepancy between K_1 and K_1 suggests the possibility that there may be another MeAIB-inhibited alanine transport system with a high affinity for MeAIB and alanine. Until we can resolve this issue we shall continue to refer to the MeAIB-inhibited portion of alanine transport as the A system. We are looking into the possibility that these systems may be separated by the relative tolerance for the substitution of Na⁺ by Li⁺ (see Fig. 8).

The system immune to MeAIB, as we have shown, has the major characteristics of the ASC system and has been referred to as such. We initially attempted to eliminate the L system from these studies by employing BCH as a possible specific inhibitor of the L system (23, 24). We found, however, that BCH, besides possibly eliminating the L system in alanine transport, also inhibited alanine transport probably through the ASC system. The ASC system turns out to be the major transport system for alanine. The V_max values are not significantly different (0.84 as compared to 0.72 mM/min).

Studies of the specificity of the ASC system by inhibition analysis of alanine uptake in the presence of MeAIB and BCH and by direct determination of that portion of the velocity of uptake of individual amino acids that is resistant to MeAIB and that is Na⁺-dependent in general gave similar results. This is in contrast to what has been reported in other studies (3, 16) where many amino acids that inhibited transport through the ASC system appeared incapable of being transported by it. The Na⁺ dependence of this system and the finding that serine, cysteine, alanine, and threonine are transported at the highest velocities as compared to the other compounds tested are all attributes of the ASC system previously characterized in Ehrlich ascites cells (2, 3). We, however, find no evidence for accelerative exchange diffusion in CHO-K1 in the transport of alanine and only weak exchange diffusion. The specificity studies have uncovered notable differences between the ASC system, as well as the A system, in CHO-K1 and those systems found in the other cell lines (3, 16). In the rat hepatocyte (see Ref. 16, Table II), it was found that alanine, serine, and methionine are mainly transported by the A system. In contrast, in CHO-K1 these amino acids are mainly transported by the ASC system with serine being apparently specific for this system. In the rat hepatocyte, cysteine is exclusively carried by the ASC system, but only 55% is transported by the ASC system in CHO-K1.

There are gross similarities between the ASC system in Ehrlich ascites cells (2, 3) and CHO-K1. In both cell lines, the ASC system is the major Na⁺-dependent system for the transport of alanine. However, there is no evidence that serine is specific for the ASC system in Ehrlich cells as it is in CHO-K1. Also, Ehrlich cells have little or no uptake attributable to the ASC system of methionine, valine, and leucine while there is significant uptake of these amino acids by the ASC system in CHO-K1. The ASC system, in both cell lines, is unable to function with Li⁺ replacing Na⁺. In this respect, ASC transport in CHO-K1 and Ehrlich ascites cells appears to differ from ASC transport in rat hepatocytes (15, 16).

Our data also indicate that AIB at 0.1 mM is transported 62% by the A system and 25% and 12% by the L and ASC systems, respectively, and again emphasize the care that should be taken before using this analogue as a specific substrate for the A system (23).

Addendum—While this work was in progress, we were informed of a similar study by M. A. Shotwell, D. W. Jayne, M. S. Kilberg, and D. L. Oxender (unpublished manuscript).

REFERENCES
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