KINETIC PARAMETERS OF NEUTRAL AMINO ACID TRANSPORT IN HYBRIDS BETWEEN MALIGNANT AND NON-MALIGNANT CELLS

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SUMMARY

The kinetics of neutral amino acid transport were examined in isogeneic matched pairs of hybrid cells, one member of each pair being tumorigenic, the other not. The L system of transport, which is sodium-independent, was measured by the uptake of leucine, and the A system, which is sodium-dependent, by the uptake of methylaminoisobutyric acid. Although there was variation from one cell type to another in both $K_m$ and $V_{max}$ for the transport of these amino acids, no systematic change was found to be associated with tumorigenicity. This is in marked contrast with hexose uptake where tumorigenicity was invariably found to be associated with a reduction in $K_m$. It thus appears that whatever molecular change is responsible for the alteration in the kinetics of hexose transport, it is specific, at least to the extent that it does not affect either sodium-dependent or sodium-independent transport of neutral amino acids.

INTRODUCTION

In previous studies (White, Bramwell & Harris, 1981, 1982, 1983) we have shown that malignancy, as defined by the ability of cells to grow progressively in vivo, is associated with a systematic reduction in the Michaelis constant ($K_m$) for hexose transport. This association was demonstrated, without exception, in 86 different cell lines, including matched pairs of hybrid cells in which one member of each pair was malignant and the other not. It was also shown that the reduction in $K_m$ was determined primarily by the transport of hexose across the cell membrane and not by its subsequent phosphorylation. In considering the various mechanisms by which this reduction in $K_m$ might be brought about, one possibility was that it might be determined by a generalized change in the cell membrane that could affect the transport of nutrients other than hexoses. This possibility was explored in the present investigation by an examination of the kinetic parameters of neutral amino acid transport in malignant and non-malignant cells. Isogeneic matched pairs of hybrid cells were again used to make the comparison.

Three major systems for the transport of neutral amino acids have been characterized in mammalian cells on the basis of their substrate specificity and their requirement for sodium ions (Oxender & Christensen, 1963; Christensen, Liang & Archer, 1967; Oxender, Lee, Moore & Cecchini, 1977). System L serves mainly for the uptake of branched chain and aromatic amino acids and is sodium-independent.

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System A serves mainly for the uptake of amino acids with short or linear side-chains; it is sodium-dependent, concentrates amino acids within the cell and is tolerant to N-methylation of the substrate. System ASC is also sodium-dependent but has a greater reactivity with alanine, serine and cysteine and is intolerant to N-methylation of the substrate. These differences in substrate specificity have been used to isolate systems A and L experimentally and thus to measure their kinetic parameters in the different cell types.

MATERIALS AND METHODS

Labelled amino acids

L-[4,5-3H]leucine was obtained from Amersham International Ltd, Bucks, U.K. and α-[1-14C]-methylamino-isobutyric acid from New England Nuclear, Boston, Mass., U.S.A. Unlabelled amino acids and analogues were from Sigma Chemical Company Ltd, Poole, Dorset, U.K. except for β-2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid which was from Calbiochem-Behring Corporation, La Jolla, Calif., U.S.A.

Choline buffer

Choline buffer contained 1 mM-MgCl₂, 2 mM-CaCl₂, 5 mM-KCl, 5 mM-glucose, 135 mM-choline chloride and 10 mM-Tris-HCl adjusted to pH 7.5.

Sodium buffer

Sodium buffer contained 1 mM-MgCl₂, 2 mM-CaCl₂, 5 mM-KCl, 5 mM-glucose, 135 mM-NaCl and 10 mM-Tris-HCl adjusted to pH 7.5.

Cell lines

Mouse

PG19: hypoxanthine-guanine phosphoribosyl transferase-deficient (HGPRT⁻) derivative of a spontaneous melanoma arising in a C57B1 mouse (Jonasson, Povey & Harris, 1977).

PG19WGAR clone C2: non-malignant derivative of PG19 resistant to 10 μg/ml of wheat-germ agglutinin (Bramwell & Harris, 1978).

PG19 x T13HT13H clone 8: non-malignant hybrid produced by fusion of PG19 and diploid fibroblasts homozygous for the T13H translocation (Jonasson et al., 1977).

PG19 x T13HT13H clone 8T1: tumour derived from clone 8 (Jonasson et al., 1977).

Human

S1814: diploid fibroblast strain from male foetus (Klinger, 1980).

DS1: diploid fibroblast strain from spontaneous male abortus (White et al., 1983).

HeLa (Flow): malignant cell line derived from carcinoma of cervix (Gey, Coffman & Kubicek, 1952) supplied by Flow Laboratories, Irvine, Scotland, U.K.


HeLa D98 F908 A3 x S1814 clone 2B1Col1: non-malignant hybrid between a HeLa derivative and human fibroblast line S1814 (Klinger, 1980).

HeLa D98 F908 A3 x S1814 clone 5Amc3: malignant hybrid between a HeLa derivative and human fibroblast line S1814 (Klinger, 1980).

HeLa D98 F908 A3 x S1814 clone 1Acm2: non-malignant hybrid between a HeLa derivative and human fibroblast line S1814 (Klinger, 1980).

HeLa D98 F908 A3 x S1814 clone 1Aen1TG: malignant hybrid between a HeLa derivative and human fibroblast line S1814 (Klinger, 1980).
Assay of leucine uptake

The activity of the L system for neutral amino acid transport is defined experimentally as the initial rate of sodium-independent leucine or methionine uptake at pH 7.5 (Boerner & Saier, 1982). The protocol described for measurement of hexose transport (White et al. 1983) was adapted to measure the initial rate of leucine uptake.

Cells were suspended in Eagle's minimal essential medium containing 10% foetal calf serum (2 x 10^5 cells per ml) and evenly distributed into 48, 16-mm diam. tissue culture wells (Costar, Cambridge, Mass., U.S.A.). A sample (1 ml) of cell suspension was added to each well 24 h before assay. The medium was aspirated from the adherent cell monolayer and replaced with choline buffer (1 ml). The cultures were then incubated for 1 h at 37°C in order to deplete endogenous pools of amino acid and washed with choline buffer (20°C). Uptake was initiated by addition of 250 μl of a solution of L-[4,5-^3H]leucine (2–100 μM, 0.2–10 mCi/μmol) in choline buffer at 20°C. The radioactive medium was aspirated after 10–30 s and the monolayer rapidly washed three times with ice-cold choline buffer (700 μl). The cell monolayers were dissolved in 440 μl of 0.4 M NaOH, neutralized with 10 μl of glacial acetic acid and then mixed with 10 ml of Unisolve 1 (Koch-light, Colnbrook, Berks, U.K.). The activity of tritium in the samples was determined in a Packard scintillation counter. In each experiment, six wells were assayed for each leucine concentration. Six different concentrations of leucine were used in most experiments.

Non-specific retention of radiolabel was measured with 10 mM-L-[4,5-^3H]leucine. Measurements were made on six wells and averaged. The method used for determining the number of cells per well and the calculations were as described for hexose uptake (White et al. 1983).

Assay of methylaminoisobutyric acid uptake

The activity of the A system for neutral amino acid transport is defined experimentally as the initial rate of sodium-dependent methylaminoisobutyric acid uptake at pH 7.5 (Boerner & Saier, 1982). The protocol for the assay of uptake was as for leucine with the following modifications: (a) sodium buffer replaced choline buffer at all stages in the procedure; (b) the substrate was α-[1-^14C]-methylaminoisobutyric acid (0.1–5 μM, 1–53.4 mCi/mmol) dissolved in sodium buffer; (c) uptake was measured for periods up to 30 min; (d) non-specific retention of radiolabel was measured by incubating the cells with 10 mM-α-[1-^14C]methylaminoisobutyric acid for short periods (<10 s).

RESULTS

Leucine uptake

Since the neutral amino acid transport system L differs from systems A and ASC in not being dependent on sodium ions, it can be isolated experimentally by the use of buffer in which choline chloride replaces sodium chloride (choline buffer). Sodium-independent leucine uptake was linear for short periods only, and time points over less than half a minute were necessary. In the range of substrate concentrations used, initial rates of leucine uptake obeyed Michaelis–Menten kinetics. The kinetic parameters of this uptake in mouse cells are given in Table 1. There is variation in both $K_m$ and $V_{max}$ between different cell types, but neither $K_m$ nor $V_{max}$ correlates with tumorigenicity. For example, the malignant melanoma PG19 and its non-malignant derivative, PG19WGARC2, differed markedly in the $K_m$ for hexose transport (White et al. 1981) but show no significant difference in the kinetic parameters of leucine uptake.

The kinetic parameters of leucine uptake by human cells are given in Table 2. Again, there is no correlation between tumorigenicity and the magnitude of either the
Table 1. *Kinetic parameters of sodium-independent leucine uptake in mouse cells*

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Tumorigenicity</th>
<th>No. of concentrations assayed</th>
<th>(K_m) ((\mu M))</th>
<th>(V_{max}) (nmol/106 cells per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG19</td>
<td>+</td>
<td>5</td>
<td>22.42 ± 3.77</td>
<td>5.74 ± 7.74</td>
</tr>
<tr>
<td>PG19 WGAR clone 2</td>
<td>-</td>
<td>6</td>
<td>21.18 ± 1.21</td>
<td>5.13 ± 1.80</td>
</tr>
<tr>
<td>C57Bl fibroblasts (pass. 5)</td>
<td>-</td>
<td>6</td>
<td>38.50 ± 2.25</td>
<td>91.42 ± 4.54</td>
</tr>
<tr>
<td>T13H/T13H fibroblasts (pass. 4)</td>
<td>-</td>
<td>5</td>
<td>20.62 ± 1.25</td>
<td>96.45 ± 5.23</td>
</tr>
<tr>
<td>PG19 X T13H/T13H fibroblast clone 8</td>
<td>-</td>
<td>5</td>
<td>12.13 ± 1.94</td>
<td>27.80 ± 2.68</td>
</tr>
<tr>
<td>PG19 X T13H/T13H fibroblast clone 8T1</td>
<td>+</td>
<td>6</td>
<td>56.17 ± 2.65</td>
<td>141.80 ± 5.5</td>
</tr>
</tbody>
</table>

Table 2. *Kinetic parameters of sodium-independent leucine uptake in human cells*

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Tumorigenicity</th>
<th>No. of concentrations assayed</th>
<th>(K_m) ((\mu M))</th>
<th>(V_{max}) (nmol/106 cells per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS1 fibroblasts (pass. 9)</td>
<td>-</td>
<td>6</td>
<td>25.05 ± 1.71</td>
<td>58.7 ± 2.8</td>
</tr>
<tr>
<td>S1814 fibroblasts (pass. 7)</td>
<td>-</td>
<td>5</td>
<td>37.32 ± 2.74</td>
<td>137.6 ± 8.0</td>
</tr>
<tr>
<td>HeLa (Flow)</td>
<td>+</td>
<td>6</td>
<td>12.18 ± 0.16</td>
<td>170.6 ± 1.2</td>
</tr>
<tr>
<td>HT29/219</td>
<td>+</td>
<td>5</td>
<td>18.17 ± 0.77</td>
<td>213.8 ± 6.9</td>
</tr>
<tr>
<td>H. Ep. 2</td>
<td>+</td>
<td>6</td>
<td>54.90 ± 9.82</td>
<td>258.1 ± 38.3</td>
</tr>
<tr>
<td>S1814 X HeLa D98 1Acm2</td>
<td>+</td>
<td>6</td>
<td>21.79 ± 1.68</td>
<td>93.2 ± 5.9</td>
</tr>
<tr>
<td>S1814 X HeLa D98 1Acm1TG</td>
<td>+</td>
<td>5</td>
<td>39.92 ± 0.86</td>
<td>435.4 ± 7.2</td>
</tr>
<tr>
<td>S1814 X HeLa D98 2BCol1</td>
<td>+</td>
<td>6</td>
<td>33.65 ± 3.20</td>
<td>212.8 ± 13.1</td>
</tr>
<tr>
<td>S1814 X HeLa D98 5Acm3</td>
<td>+</td>
<td>6</td>
<td>42.31 ± 5.45</td>
<td>320.2 ± 35.4</td>
</tr>
</tbody>
</table>

\(K_m\) or \(V_{max}\) of uptake. For example, in the hybrids derived from the fusion of diploid human fibroblasts with HeLa cells (S1814 X HeLa D98, Klinger, 1980), a very strong correlation was found between tumorigenicity and the \(K_m\) of hexose transport, but these same cells have very similar Michaelis constants for leucine transport.

*Methylaminoisobutyric acid uptake*

The active transport of methylaminoisobutyric acid across the plasma membrane is largely confined to system A (Christensen et al. 1967), since this system is tolerant to \(N\)-methylation of substrate. Preliminary experiments showed that uptake of methylaminoisobutyric acid by HeLa(Flow) cells had the following characteristics: (1) it was linear for at least 30 min; (2) it was reduced to 5% of the control value if sodium chloride was replaced by choline chloride in the assay solutions; (3) it was inhibited by 80% by unlabelled methylaminoisobutyric acid, aminoisobutyric acid,
Neutral amino acid transport in hybrid cells

Table 3. Kinetic parameters of sodium-dependent methylaminoisobutyric acid uptake in human cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Tumorigenicity</th>
<th>No. of concentrations assayed</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (nmol/10^6 cells per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS1 fibroblasts (pass. 8)</td>
<td>-</td>
<td>4</td>
<td>13.17 ± 2.75</td>
<td>7.64 ± 1.47</td>
</tr>
<tr>
<td>S1814 fibroblasts (pass. 7)</td>
<td>-</td>
<td>6</td>
<td>0.303 ± 0.035</td>
<td>6.98 ± 0.21</td>
</tr>
<tr>
<td>HeLa (Flow)</td>
<td>+</td>
<td>5</td>
<td>0.606 ± 0.051</td>
<td>2.47 ± 0.10</td>
</tr>
<tr>
<td>H.Ep.2</td>
<td>+</td>
<td>4</td>
<td>0.802 ± 0.053</td>
<td>2.77 ± 0.13</td>
</tr>
<tr>
<td>S1814 × HeLa D98 2B1Coll</td>
<td>-</td>
<td>5</td>
<td>0.950 ± 0.131</td>
<td>35.78 ± 3.19</td>
</tr>
<tr>
<td>S1814 × HeLa D98 5Amc3</td>
<td>+</td>
<td>6</td>
<td>0.634 ± 0.010</td>
<td>5.31 ± 0.04</td>
</tr>
</tbody>
</table>

alanine and N-methylalanine, but only inhibited by 20 to 30% by β-2-aminobicyclo-(2,2,1)-heptane carboxylic acid, leucine, valine and N-methylvaline (each present at 10 mM); (4) it was stimulated two- to ten-fold by 5-methoxy-N,N-dimethyltryptamine, and this effect was sodium-dependent. This compound also stimulated uptake of 22Na⁺ into DS1 human fibroblasts (unpublished data) suggesting that the unexpected stimulation of amino acid uptake was due to an alteration in the sodium balance across the cell membrane rather than a direct effect on the amino acid transport system itself.

These data confirm that methylaminoisobutyric acid uptake is largely confined to system A.

Table 3 gives the kinetic parameters of methylaminoisobutyric acid uptake for several cell lines. Again, there is variation between different cell lines. For example, the $K_m$ of uptake of DS1 human fibroblasts is remarkably high compared to the other cell lines. However, there is no correlation between either $K_m$ or $V_{max}$ and tumorigenicity.

DISCUSSION

The present study demonstrates that although there is variation from one cell type to another in the kinetic parameters of amino acid transport, no systematic change in these parameters is associated with tumorigenicity. This is in marked contrast with hexose transport where tumorigenicity was invariably found to be associated with a reduction in $K_m$. It thus appears that whatever molecular change is responsible for the alteration in the kinetics of hexose transport, it is specific at least to the extent that it does not affect either sodium-dependent or sodium-independent transport of neutral amino acids.

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REFERENCES


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