Transport of Amino Acid Amide Sarcosinamide and Sarcosinamide Chloroethylnitrosourea in Human Glioma SK-MG-1 Cells

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ABSTRACT

The transport of the amino acid amide N\(^{1}\)H\(^{3}\)H\(^{1}\)sarcosinamide (methyl glycynamide) was investigated in human glioma SK-MG-1 cells. Sarcosinamide uptake was found to be temperature dependent, sodium independent, and linear up to 1 min at 22°C. Equilibrium was reached after 10 min at 22°C with accumulation slightly above unity. Sarcosinamide was not metabolized in the cells as shown by thin layer chromatography. The uptake of sarcosinamide was significantly decreased when the extracellular pH was lowered from 7.5 to 6.0 and significantly elevated at pH values above 7.5. The latter effect may be due to a mixture of increased cell permeability at high pH. The uptake of the labeled sarcosinamide was trans-stimulated by excess cold sarcosinamide. Sarcosinamide uptake over a 200-fold range of concentrations followed Michaelis-Menten kinetics with a \(K_m\) of 0.284 ± 0.041 \(\mu\)M and a \(V_{max}\) of 0.154 ± 0.024 nmol/10\(^6\) cells/min. The uptake of sarcosinamide was significantly reduced by iodoacetate but not by the metabolic poisons NaF, ouabain, or dinitrophenyl, suggesting that the uptake is not dependent on energy, rather it proceeds by facilitated diffusion. Several naturally occurring substrates were unable to inhibit the uptake of sarcosinamide. Leucine significantly reduced the uptake of sarcosinamide, while sarcosinamide was a weak inhibitor of leucine transport. 2-Aminobicyclo[2,2,1]heptane-2-carboxylic acid, a specific substrate for the sodium-independent, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid-sensitive amino acid system L failed to inhibit the uptake of sarcosinamide. Epinephrine reduced the uptake of sarcosinamide and sarcosinamide was equally potent as an inhibitor of epinephrine transport. Dixon plot analysis demonstrated that epinephrine \((K_m = 0.270 \mu\)M) inhibits the uptake of sarcosinamide competitively \((K_c = 0.260 \mu\)M). These results indicate that sarcosinamide is a substrate for the catecholamine transporter. The alkylating agent, sarcosinamide chloroethylNitrosourea, was tested for its ability to inhibit the uptake of sarcosinamide. The results of Dixon plot analysis were consistent with competitive inhibition of sarcosinamide uptake and the inhibition constant \(K_i\) for SarCNU was found to be 3.26 ± 0.57 \(\mu\)M. The steady-state intracellular concentration of SarCNU was found to be significantly higher (cell:medium ratio of 1.03 ± 0.01) than that of BCNU (cell:medium ratio of 0.52 ± 0.12). These findings indicate that SarCNU and sarcosinamide share the same carrier for uptake in SK-MG-1 cells. This transport mechanism may be responsible for the increased accumulation of SarCNU as compared to BCNU, a nitrosourea which enters cells by passive diffusion.

INTRODUCTION

Sarcosinamide chloroethylNitrosourea is a compound with the amino acid amide group, sarcosinamide, which is the amide derivative of methylglycine (1) (Fig. 1). SarCNU\(^4\) was previously shown to exhibit increased \(in vitro\) toxicity in primary and established human glioma cells as compared to BCNU, the standard agent of choice in the chemotherapy of brain tumors (2). SarCNU was also more active than BCNU in nude mice bearing intracerebrally implanted human glioma cells (3). Finally, SarCNU is less toxic in mice and exhibits less \(in vitro\) myelotoxicity than BCNU (1, 4). The presence of an amino acid amide on SarCNU led to the speculation that its transport may differ from that of BCNU which enters cells by passive diffusion (5), and that this may possibly account for the observed difference in the cytotoxic effect between these two compounds in glioma cells.

This hypothesis was initially tested by indirect methods patterned after the experiments of Vistica (6) who used cytotoxicity as an indicator for the transport of the antitumor agent, melphalan. The cytotoxicity of SarCNU in the human glioma SKMG-1 cells was not reduced in media containing severalfold excess amounts of amino acids. However, in the presence of excess concentrations of sarcosinamide, the cell-killing activity of SarCNU was significantly reduced. Furthermore the uptake of tritiated sarcosinamide was significantly inhibited by excess unlabeled sarcosinamide and excess SarCNU, indicating the existence of a carrier-mediated uptake of sarcosinamide in the SK-MG-1 cells which may also accommodate SarCNU (7).

Tritiated sarcosinamide was utilized in the present study to further define this transport. Two considerations prompted the use of sarcosinamide in this study. First, SarCNU is not available in a radiolabeled form. Second, it is expected that sarcosinamide is the native substrate for this transport and would, therefore, be more suitable than SarCNU for its characterization. The effect of various physiological inhibitors, metabolic inhibitors, and pH on the uptake of sarcosinamide was examined in the SK-MG-1 cells. The kinetic parameters \((K_m\) and \(V_{max}\)) for sarcosinamide were defined. The method of Dixon (8) was used to determine whether SarCNU inhibits the transport of sarcosinamide in a competitive fashion and to obtain the inhibition constant, \(K_i\), for SarCNU. Finally, the intracellular concentrations of SarCNU and BCNU were measured in SK-MG-1 cells under steady-state conditions.

MATERIALS AND METHODS

Drugs. SarCNU (NSC 364432) was a gift from Dr. T. Suami, Keio University, Japan. BCNU was supplied by the Drug Development Branch, National Cancer Institute, Bethesda, MD. The drugs were dissolved in 0.001 M citrate buffer, pH 4.0, and stored at −20°C.

Materials. Sarcosinamide HCl, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride (Britton-Marshall reagent), glycylglycine HCl, sulfosalicylic acid, (\(\pm\))-epinephrine HCl, and all unlabeled amino acids were obtained from Sigma Chemical Co. N\(^{\text{14}}\)N\(^{\text{15}}\)HN-sarcosinamide hydrochloride (1.05 Ci/mmol) was prepared by Amersham Laboratories, Buckinghamshire, England, utilizing a catalyzed exchange procedure with high specific activity tritiated water. The radiochemical purity was 96%, using thin layer chromatography on cellulose in butan-1-ol:water:acetic acid (12:5:3) or ethanol:0.88 ammonium water (80:4:16) with \(R_f\) values of 0.34 and 0.68, respectively. L-[\(\text{\textsuperscript{14}}\)C]Leucine (330 M Ci/mmol), [carboxyl\(^{13}\)C]Glycine (3.2 M Ci/mmol) and tritiated water (90 M Ci/mmol) were all purchased from Amersham Corp. L-[\(\text{\textsuperscript{13}}\)C]Epinephrine (66.8 M Ci/mmol) was purchased...
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Fig. 1. Chemical structures of SarCNU (a) and sarcosinamide (b).

from NEN. BCH, isomeric form b(+), was purchased from CalbiochemBehring Corp. 2,4-Dinitrophenol, iodoacetate, and ouabain were purchased from Aldrich. McCoy's modified medium 5A, fetal calf serum, and Dulbecco's phosphate-buffered saline were supplied by Grand Island Biological Co., Grand Island, NY; bovine serum albumin (Fraction V, powder; low-salt and salt-free fractions) by Miles Laboratories; Versilube F-50 silicone oil was from Nessa Products; choline chloride and dextrose were from Fisher Laboratories.

Cells. The SK-MG-1 cells were derived from a previously untreated human glioma biopsy and were a kind gift from Dr. G. Cairncross, University of Western Ontario, London, Ontario, Canada. The cells were grown and maintained in McCoy's medium 5A supplemented with 10% fetal calf serum and 4 μg/ml gentamycin (Shering, Pointe Claire, Quebec, Canada) in a humidified 5% CO2 atmosphere at 37°C. The cells were found to be free of Mycoplasma with the Hoechst stain kit, Flow Laboratories, Mississauga, Ontario, Canada.

Transport Experiments. Two methods were used to assay the uptake of sarcosinamide in the SK-MG-1 cells. In the first method, transport was performed on cells in suspension. Cells were suspended in PAG at 4 x 10^6 cells/ml. Tritiated sarcosinamide at various concentrations was also prepared in PAG. Transport assays were also carried out in the absence of sodium by replacing this ion in PAG by an equivalent quantity of sarcosinamide solution to an equal volume of the SK-MG-1 cells. At indicated time points, 400-μl aliquots of the reaction mixture were layered onto 1 ml of Versilube oil and the reaction was terminated by centrifugation at 12,000 x g for 1 min at 22°C in a Brinkman-Eppendorf microcentrifuge to separate cells from the medium as previously described (6). Cell pellets were solubilized in 0.1 ml of 0.6 M perchloric acid. The solubilized material was then centrifuged at 12,000 x g for 4 min to pellet cellular macromolecules. The supernatants were placed in 10 ml Scintiverse and counted in a Wallac 1217 Rackbeta liquid scintillation spectrophotometer and corrected for quenching.

The ICW was determined with tritiated water and [carboxyl-14C]-inulin by centrifugation through Versilube oil to separate the medium from the cells as previously described (9). Uptake of sarcosinamide was expressed as the cell:medium ratio which describes the distribution of the radiolabel in the ICW and the extracellular medium.

In the second method, transport of sarcosinamide was assayed on confluent monolayers of SK-MG-1 cells (1 x 10^6 cells/35-mm plate) at 22°C according to the technique described by Ronquist et al. (10). McCoy's medium was removed by aspiration and the cells were washed once with 2 ml of phosphate-buffered saline pH 7.4, and 0.1% bovine serum albumin. Following a 15- to 30-min preincubition of the cells in PAG or unlabeled 25 mM sarcosinamide, the transport was initiated by the addition of 1 ml of N-[3H]sarcosinamide alone or with various concentrations of osmotically adjusted physiological inhibitors in PAG. For trans-stimulation experiments, the external medium (PAG or PAG plus 25 mM sarcosinamide) was aspirated and the cells were then rinsed 3 times with PAG at 22°C. This procedure took less than 6 s. The cells were then exposed to N-[3H]sarcosinamide for 30 s. At indicated time points, the permeant solutions were rapidly aspirated and the cells were rinsed 5 times with 2 ml ice-cold phosphate-buffered saline supplemented with 48 mM glycylglycine. Glycylglycine was included in the rinsing buffer because it removes radiolabel bound to the plastic surface of the Petri dish. It took 10 s to complete this step and the cells remained attached to the plates throughout this procedure. The cells were then solubilized with 2 ml of 3% sulfosalicylic acid at 60°C for 10 min, the extracts were transferred to borosilicate tubes and heated to 100°C for 5 min. Following centrifugation at 300 x g to pellet cellular debris, 800-μl aliquots of the supernatant were put in 10 ml Scintiverse liquid and counted as described above. The number of cells per 35-mm plate was determined by counting cells in 6 plates on a Coulter Counter and using the average as the cell number per plate. The mean of the cell counts obtained by this method has a standard error of less than or equal to 5%. The uptake of sarcosinamide was expressed as nmol/10^6 cells. This method was also used to examine the transport of radiolabeled epinephrine and leucine in the SK-MG-1 cells.

Metabolism of N-[3H]Sarcosinamide. Samples obtained from transport assays done on cells in suspension were utilized in these experiments. The cells had been incubated with N-[3H]sarcosinamide for 10 min at 22°C and separated from the permeant as described above. The cell contents were analyzed for the presence of intact sarcosinamide by thin layer chromatography on No. 13255 cellulose plates (Kodak), using butan-1-ol-water:acetic acid (12:5:3). In this system intact sarcosinamide has an Rf of 0.34.

Measurements of Steady-State Levels of BCNU and SarCNU. The method used was a modified version of the technique described by Loo and Dion (11). These experiments were done with SK-MG-1 cells in suspension (2 x 10^6 cells/ml PAG). Following a 15-min preincubation at 37°C, the cells were exposed to 1 mM BCNU or SarCNU for 30 min at 37°C (concentrations below 1 mM would exceed the limit of sensitivity in this assay). The reaction mixtures were distributed into ten 400-μl aliquots and layered onto 1 ml of Versilube oil. The cells were separated from the medium by centrifugation as described above, the cell pellets were solubilized in 50 μl of 0.5% sulfinamide in 1 N HCl, and the cellular extracts were combined (0.5 ml final volume). Standards of BCNU and SarCNU as well as 10-μl aliquots of the reaction media were each treated with 0.5 ml of 0.5% sulfinamide in 1 N HCl. The extracts were incubated at 50°C for 45 min, transferred to an ice bath, and treated with 0.1 ml of the Britton-Marshall reagent for 10 min at 22°C. The absorbance was determined at 540 nm on a Hewlett-Packard 8451A diode array spectrophotometer and the μg of intracellular CNU was evaluated by extrapolation from standard curves. The concentration of BCNU or SarCNU was obtained as μg/μl ICW which was determined as described above. The steady-state levels of these compounds were expressed as cell:medium ratios.

Calculations. Kinetic parameters were determined by linear regression analysis of Lineweaver-Burk plots. Statistical analysis was performed by the two-tailed t test.

RESULTS

Time Course of Uptake of N-[3H]Sarcosinamide. A time course of uptake of 0.05 mM sarcosinamide in SK-MG-1 cells in suspension is shown in Fig. 2 at 0 and 22°C (the uptake of this amino acid amide at 37°C was too rapid, therefore all subsequent studies were done at 22°C). The intracellular water space was found to be 1.12 ± 0.133 μl/10^6 cells. The specific activity of the tritiated sarcosinamide was 9.9 μCi/μmol. The uptake of sarcosinamide after 1 min was found to be 23 ± 2.65 pmol/10^6 cells at 22°C. The uptake at 22°C was linear up to 1 min and equilibrium was observed after 10 min with the cell:medium ratio of 1.2. The uptake of sarcosinamide was reduced at 0°C.

Fig. 2. Time course of the uptake of 0.05 mM N-[3H]sarcosinamide by SK-MG-1 cells in suspension at 22°C (□) and at 0°C (■). The uptake is expressed as cell:medium distribution ratio as described in "Materials and Methods." Points, mean of four separate experiments; bars, SE.
All subsequent experiments were terminated at 30 s to approximate this initial rate of uptake conditions.

**Metabolism of \( \text{N}-[^3\text{H}]\text{Sarcosinamide} \).** Analysis by thin layer chromatography of cellular samples following a 10-min incubation with 0.05 mM \( \text{[^3\text{H}]Sarcosinamide} \) resulted in an R\(_f\) value which was similar to that of intact sarcosinamide. This suggests that sarcosinamide is not metabolized in the SK-MG-1 cells.

**Effect of Sodium on Transport of \( \text{N}-[^3\text{H}]\text{Sarcosinamide} \).** The cell:media ratios at 30 s following an incubation with 0.05 mM \( \text{[^3\text{H}]Sarcosinamide} \) in PAG and in sodium-free media were found to be 0.50 ± 0.09 and 0.60 ± 0.02, respectively. These findings indicate that the uptake of sarcosinamide is not dependent upon sodium.

**Trans-stimulation of Sarcosinamide Uptake in SK-MG-1 Cells.** The effect of intracellular sarcosinamide on the initial uptake of \( \text{N}-[^3\text{H}]\text{Sarcosinamide} \) into the SK-MG-1 cells on plates was examined by comparing the transport of 0.05 mM of this amino acid amide (specific activity, 9.9 \( \mu \)Ci/\( \mu \)mol) into cells preloaded with either PAG or 25 mM sarcosinamide in PAG for 30 min at 22°C. The uptake at 30 s of \( \text{[^3\text{H}]Sarcosinamide} \) into cells preloaded with PAG was found to be 0.017 ± 0.004 nmol/10\(^6\) cells. The uptake into cells preloaded with sarcosinamide was significantly increased to 0.032 ± 0.008 nmol/10\(^6\) cells \((P < 0.05)\), suggesting the involvement of a carrier for the uptake of sarcosinamide into the SK-MG-1 cells.

**Kinetics of Sarcosinamide Uptake.** The 30-s uptake of sarcosinamide was examined for concentrations from 0.005 to 10 mM. The velocity of sarcosinamide uptake is plotted as a function of its concentration (0.005-1.0 mM) in Fig. 3. The rate of sarcosinamide transport into SK-MG-1 cells follows Michaelis-Menten kinetics with saturation of transfer evident when the concentration of sarcosinamide is increased to 0.5 mM. Fig. 4 shows a Lineweaver-Burk plot for sarcosinamide uptake between 0.005 and 1.0 mM. The \( \text{Km} \) was found to be 0.284 ± 0.041 mM and the \( \text{Vmax} \) 0.154 ± 0.024 nmol/10\(^6\) cells/min.

**Dixon Plot Analysis of Sarcosinamide and SarCNU Transport.** Fig. 5 shows a representative Dixon plot of the effect of increasing levels of external SarCNU on the uptake of 0.050, 0.075, and 0.100 mM of tritiated sarcosinamide. The point of intersection of the three resultant plots is consistent with competitive inhibition of sarcosinamide uptake by SarCNU and yields a \( \text{Ki} \) of 4.35 mM for SarCNU in this experiment. The mean \( \text{Ki} \) determined from 4 separate experiments was found to be 3.26 ± 0.57 mM. The differences in the mean between the above \( \text{Ki} \) and the \( \text{Km} \) obtained for sarcosinamide were significant \((P < 0.05)\).

**Effect of External pH.** The initial uptake rates of sarcosinamide at pH values between 5.5 and 9.0 are shown in Fig. 6. The pH of the permeant solution was adjusted with HCl or NaOH immediately prior to addition to the mono-layers of SK-MG-1 cells. The uptake of 0.01 mM \( \text{[^3\text{H}]Sarcosinamide} \) at 22°C at external pH in the range of 5.5-9.0 was examined as described in “Materials and Methods.” The uptake is expressed as the percentage of the uptake velocity at pH 7.5. The uptake at pH 7.5 was 14.9 ± 1.56 pmol/10\(^6\) cells. Points, mean of three independent determinations. The plots from top to bottom represent 0.050 to 0.100 mM sarcosinamide.
leaky at higher pH, the uptake of [carboxyl-14C]inulin, to which intact cells are impermeable, was measured in the SK-MG-1 cells at pH values of 7.5, 8.5, and 9.0 as described in "Materials and Methods." The uptake of [carboxyl-14C]inulin doubled at pH 8.5 and was found to be 2.5 times higher at pH 9.0 as compared to that of pH 7.5, confirming that the integrity of the cellular membrane is affected at pH values in excess of 7.5 (results not shown).

Chemical Specificity of Sarcosinamide Transport. Various naturally occurring compounds were tested as potential inhibitors of sarcosinamide transport. Excess concentrations (≥10 mM) of glucose, choline, or glucosamine failed to inhibit the uptake of this amino acid amide.

Amino acids or amino acid analogues specific for different amino acid systems were tested as inhibitors of sarcosinamide transport. Excess concentrations (10 mM) of lysine and arginine (y+ system), threonine (ASC system), methyl α-aminoisobutyric acid (A system), glycine and sarcosine (Gly system), did not decrease the uptake of 0.05 mM N-[3H]sarcosinamide. Leucine, which is transported by the L system, significantly inhibited the uptake of sarcosinamide when present at a 200-fold excess. This prompted an examination of the effect of BCH on sarcosinamide transport and the effect of sarcosinamide on the transport of leucine. The results are summarized in Table 1. BCH, which is a specific substrate of the Na+-independent L system (12) did not significantly inhibit the uptake of sarcosinamide, while sarcosinamide was only a weak inhibitor of leucine uptake. The weak inhibition of leucine uptake by sarcosinamide suggested that a small component of leucine transport may proceed via the sarcosinamide carrier. A subsequent Dixon plot analysis of sarcosinamide transport in the presence of leucine did not provide conclusive evidence for competitive inhibition and therefore it is unlikely that leucine is a substrate for this uptake (results not shown).

The catecholamine, epinephrine, was next examined as a potential inhibitor of sarcosinamide uptake. Epinephrine was chosen because based on previous descriptions about extraneuronal catecholamine uptake (13–15), it was found to have transport-related similarities with sarcosinamide. The results of inhibition experiments with the two compounds are summarized in Table 2. Excess epinephrine causes a significant inhibition of sarcosinamide transport and, in a reverse experiment, sarcosinamide is an equally potent inhibitor of epinephrine. The outcome of Dixon plot analysis (Fig. 7) confirms that epinephrine is a competitive inhibitor of sarcosinamide transport with a Ki value of 0.260 mM. The Km value for epinephrine uptake in SK-MG-1 cells was found to be approximately 0.270 mM (results not shown). These findings suggest that sarcosinamide and epinephrine have similar affinities for a common carrier.

Table 1 Inhibition studies with N-[3H]sarcosinamide and [14C]leucine
<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Uptake velocity (pmol/106 cells/min)</th>
<th>% of control uptake</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. None (N-[3H]sarcosinamide)</td>
<td>20.6 ± 2.4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10 mM leucine</td>
<td>12.2 ± 2.6</td>
<td>59</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>10 mM BCH</td>
<td>18.8 ± 2.8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>B. None ([14C]leucine)</td>
<td>416 ± 25</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2 mM sarcosinamide</td>
<td>372 ± 33.6</td>
<td>87</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>2 mM BCH</td>
<td>67.8 ± 3.1</td>
<td>16</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mean ± SE of at least four experiments.
* Two-tailed t test.
* NS, not significant.

Table 2 Inhibition studies with N-[3H]sarcosinamide and [14C]epinephrine
<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Uptake velocity (pmol/106 cells/min)</th>
<th>% of control uptake</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. None (N-[3H]sarcosinamide)</td>
<td>21 ± 0.4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10 mM epinephrine</td>
<td>13.6 ± 0.8</td>
<td>65</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B. None ([14C]epinephrine)</td>
<td>17.6 ± 2.0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10 mM sarcosinamide</td>
<td>11.6 ± 1.8</td>
<td>66</td>
<td>&lt;0.025</td>
</tr>
</tbody>
</table>

* Mean ± SE of at least four experiments.
* Two-tailed t test.

Fig. 7. Dixon plot of epinephrine inhibition of sarcosinamide transport. The 30-s uptakes of 0.025 and 0.050 mM N-[3H]sarcosinamide in media containing 0.05 to 1 mM unlabeled epinephrine were measured in SK-MG-1 cells in monolayers at 22°C as described in "Materials and Methods." Points, mean of three independent determinations. The plots from top to bottom represent 0.025 and 0.050 mM sarcosinamide, respectively.

Table 3 Effect of metabolic inhibitors on the transport of sarcosinamide
<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mM)</th>
<th>% of control uptake</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-Dinitrophenol</td>
<td>0.4</td>
<td>118 ± 28</td>
<td>4</td>
</tr>
<tr>
<td>NaF</td>
<td>2.0</td>
<td>93 ± 23</td>
<td>4</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>2.0</td>
<td>65 ± 11</td>
<td>4</td>
</tr>
<tr>
<td>Ouabain</td>
<td>0.4</td>
<td>81 ± 21</td>
<td>4</td>
</tr>
</tbody>
</table>

* The uptake of 0.01 mM sarcosinamide was 1.32 ± 0.09 pmol/106 cells.
* Significantly different from controls (P < 0.05) by the two-tailed t test.

Effect of Metabolic Inhibitors on Uptake of Sarcosinamide. Table 3 shows the results obtained when various metabolic poisons were tested for their ability to inhibit the initial uptake of tritiated sarcosinamide in the SK-MG-1 cells. 2,4-Dinitrophenol, NaF, and ouabain did not affect the initial uptake of sarcosinamide in the cells. Iodoacetate significantly reduced the initial uptake of sarcosinamide to 65% of control (P < 0.05).

Steady-State Levels of BCNU and SarCNU in SK-MG-1 Cells. The concentrations of BCNU and SarCNU attained at equilibrium in SK-MG-1 cells were compared. The cell:medium ratio for SarCNU was found to be 1.03 ± 0.01, which was significantly higher (P < 0.05) than the cell:medium ratio of 0.52 ± 0.12 obtained for BCNU. The accumulation of BCNU in the glioma cells is comparable to the cell:medium ratio of 0.2 to 0.6 reported by Begleiter et al. (5) for [14C]BCNU in L5178Y lymphoblasts.

DISCUSSION

The objective of the present study was to define the transport of the experimental antitumor agent SarCNU. Sarcosinamide which was previously proposed as the primary substrate for...
SarCNU transport (7) was utilized to address this question. The uptake of sarcosinamide in the SK-MG-1 cells was found to be temperature dependent, sodium independent, saturable and trans-stimulated. These features are consistent with a carrier-mediated transport for sarcosinamide. The finding that the accumulation of sarcosinamide does not proceed against a concentration gradient (cell:medium ratio was close to unity) suggests that this compound enters cells by facilitated diffusion. This type of transport mechanism is also suggested by the failure of metabolic poisons to inhibit the uptake of sarcosinamide, with the exception of iodoacetate. Iodoacetate is a sulfhydryl-reactive inhibitor of glycolysis. It is unlikely that glycolysis is needed as an energy source for the maintenance of sarcosinamide transport since NaF, which is also an inhibitor of glycolysis, did not reduce sarcosinamide uptake. Rather, iodoacetate may reduce the transport of sarcosinamide by reacting with the carrier.

The effect of lowered external pH on the uptake of sarcosinamide can be explained in terms of its structure. At low pH, the amino group on sarcosinamide would be protonated, resulting in a net positive charge. The decreased uptake suggests that sarcosinamide is transported predominantly as an uncharged species. The observed increase in the uptake of sarcosinamide at high pH values appears to be a result of damage to the cellular membrane. The observation that cells which are incubated in alkaline media are more permeable to inulin supports this explanation.

The chemical specificity of sarcosinamide transport was initially considered in terms of its structural similarity to amino acids. It is unlikely that sarcosinamide is transported by the L system to any significant degree. This conclusion is supported by the following findings. (a) The reduction of sarcosinamide uptake by leucine was only detectable at high concentrations (10 mM) of this amino acid; (b) sarcosinamide did not have a similar inhibitory action on leucine; (c) BCH did not decrease sarcosinamide transport. None of the other amino acid systems tested recognized sarcosinamide as a substrate for uptake. The results obtained are in agreement with our previous observation that the cytotoxicity of SarCNU in SK-MG-1 cells is not decreased in the presence of amino acids (7).

Other naturally occurring compounds which tested negative as inhibitors of sarcosinamide transport include glucose, glucoamine, and choline. The catecholamine, epinephrine, was found to inhibit the transport of sarcosinamide in a competitive manner with K, which is similar to the K, values determined for sarcosinamide and for epinephrine. These results are consistent with a common transport for epinephrine and for sarcosinamide. A catecholamine transport similar to the one outlined for sarcosinamide has been described in a variety of tissues in the central and the peripheral nervous system (13-15). This transport is saturable, sodium independent, and the K, values obtained for epinephrine or norepinephrine range from 0.002 to 0.250 mM, depending on the type of tissue studied. The uptake by this transport is greatly enhanced when the hydrogens of the catecholamine amino group are substituted by hydrocarbon groups. Both sarcosinamide and epinephrine bear such a moiety, the N-methyl group. On the basis of the above, we conclude that sarcosinamide is transported in SK-MG-1 cells by a process whose physiological function is transport of catecholamines.

The results of this study provide evidence that SarCNU uptake into SK-MG-1 cells is mediated by the same carrier which functions for the transport of sarcosinamide. The affinity of SarCNU for this uptake is lower than that for sarcosinamide as reflected by its inhibition constant, which at 3.26 mM is about 11-fold higher than the K, of sarcosinamide and the K, of epinephrine. A decreased affinity of SarCNU transport was anticipated due to the proximity of the chloroethylnitrosourea group to the putative N-methyl group of sarcosinamide. SarCNU is not the first antitumor agent which utilizes a transport for neurotransmitters. Another antitumor agent, nitrogen mustard, is transported into cells by the choline carrier (16). The existence of a carrier-mediated transport may lead to an increased intracellular level of SarCNU since the steady-state concentration of this compound in SK-MG-1 cells was found to be about 2-fold higher as compared to BCNU, a drug which accumulates by simple diffusion. The increased levels of SarCNU may partially account for its enhanced cytotoxicity to glioma cells relative to BCNU. Since catecholamine transport is found predominantly in tissues of the nervous system, SarCNU may be more selective than BCNU for chemotherapy of brain tumors and less toxic to bone marrow and other normal tissues which are targets of nitrosourea toxicity.

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