Transport of a Nonphosphorylated Nucleoside, 5′-Deoxyadenosine, by Murine Leukemia L1210 Cells*

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The mode of transport of a nonphosphorylated adenosine analog, 5′-deoxyadenosine, was studied in murine leukemia L1210 cells. This compound is not subject to the action of intracellular nucleoside-trapping kinases, and its transport can be examined without regard for effects of experimental conditions on kinase activity. Accumulation of 5′-deoxyadenosine was rapid, and nonconcentrative, with equilibrium attained within 12 s at 37°C. Kinetic studies were carried out at 20°C. We found both a nonmediated (diffusion) and a mediated transport process. The latter had an apparent $K_m$ of 115 μM, $V_{max} = 105$ pmol/10⁶ cells/min. Uptake of 5′-deoxyadenosine was inhibited by several heterologous nucleosides including adenosine, 2′-deoxyadenosine, thymine riboside, and inosine. Like 2′-deoxyadenosine, 5′-deoxyadenosine was more lipid-soluble than adenosine (from octanol/water partition studies). Compared with 5′-deoxyadenosine, adenosine had a much lower apparent $K_m$ (5 μM) and a higher $Q_{10}$ over the 27-37°C range (3.0 versus 1.3). Data obtained with adenosine might, however, reflect properties of intracellular adenosine kinase interacting with a transport process.

Transport of nucleosides across a cell membrane is often measured by determining the rate of incorporation of an endogenously supplied radioactive compound into nondiffusible intracellular material representing nucleotides + nucleic acid (1-7). In cultured animal cells, it is generally assumed that the rate of transport, rather than of intracellular phosphorylation, is rate-limiting (1). Recent evidence has suggested that the apparent kinetics of certain transport processes are affected by properties of nucleoside kinases (8, 9). Furthermore, effects of different drugs on the nucleoside kinases might be taken to represent alteration of transport processes unless appropriate methods for delineation of transport versus phosphorylation were available.

The use of low temperatures to minimize kinase activity might permit measurement of kinetics of transport alone, but many kinases are functional even near 0°C (10-12). The use of

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high cell densities without alteration of pH. Without change in any result reported here, we could also substitute a simpler medium (TES-E) containing buffered salts (15), made up to approximate the ionic environment of Ehrlich ascites tumor cells in vivo (16). The usual cell density was 7 x 10^5/200 μl.

**Uptake Studies** — Accumulation of nucleosides was measured using 200 μl of cell suspension + 5 μl of labeled substrate appropriately diluted with carrier after evaporation of ethanol. The temperature of each experiment is specified. After a measured interval, we added 600 μl of a solution containing 2 mM MgCl₂, 1.2 mM NaF, and 100 mM NaCl which essentially stopped all movement of nucleoside across the cell membrane in either direction (17, 18). In some experiments, 600 μl of a 0.1 mM solution of persantin in 150 mM NaCl was substituted. The cells were then collected by centrifugation (30 s, 100 x g) and washed once in the HgCl₂/NaI solution, and the pellets were dispersed for determination of intracellular radioactivity by liquid scintillation counting. Our method for estimation of intracellular space has been described (19).

The measurement of the mediated and nonmediated (diffusion) components of nucleoside transport were estimated as described in Ref. 20, using a Hewlett-Packard programmable calculator.

**Competition Studies** — These were carried out as described above, except that mixtures of radioactive nucleoside (18 μM final concentration) + nonradioactive nucleoside (20 mM final concentration) were employed. Total volume of addition was 10 μl or less. These studies were usually carried out at 20° for 18 s.

**Nucleoside Exudate** — This was measured by preloading cells with a 5 min incubation at 0° in medium containing 150 μM radioactive 5'-deoxyadenosine; the cells were then collected by centrifugation and suspended in fresh medium (5 x 10^5 cells/ml) at 0°, 15°, or 37°. At measured intervals, 200-μl portions of this suspension were removed and pipetted into 600 μl of HgCl₂/NaI solution described above. The cells were then collected by centrifugation and cellular radioactivity was determined.

**Chromatography** — Extracts of cell pellets were prepared, as described in Ref. 14, and chromatographed on TLC (cellulose) in Solvent C: 70% ethanol, 30% 1 M ammonium acetate. This separated 5'-deoxyadenosine from a radioactive derivative, formed by L1210 cells.

**Enzyme Assays** — Levels of intracellular kinases were measured in untreated cell pellets disrupted by freeze-thawing. A high speed supernatant fraction was obtained, and incorporation of radioactive nucleoside into nucleotides was determined using the DEAE-disc assay (21). Adenosine deaminase was measured as described in Ref. 22.

**Partition Ratios** — The partition of nucleosides between 2-octanol and an aqueous solution of 140 mM NaCl + 10 mM phosphate buffer, pH 7, was determined. The phases were equilibrated at the same temperature, and 500 μl of each phase was removed and mixed. To the aqueous (lower) layer was added 2 μl of a 5 mM solution of radioactive nucleoside containing 10^6 dpm. The phases were mixed, and the 500 μl of each was removed for measurement of radioactivity. A 200-μl portion of the lower phase was then removed, and redissolved in a fresh 200-μl portion of upper phase, to ensure that the previous determination was not influenced by presence of radioactive impurities.

**RESULTS**

**Properties of Nucleosides** — The purified preparation of radioactive 5'-deoxyadenosine was stable in 50% ethanol at -70° for at least 3 months. An initial radiopurity level of 98% had fallen to 96% over this interval. The major impurity apparently represents the result of a reaction liberating a fragment from the nucleoside molecule; a brief treatment of radioactive 5'-deoxyadenosine with 1 mM HCl produced a labeled product with the same Rf as the major impurity described above.

The water-octanol partition ratios of 5'-deoxyadenosine was 4.02.1. A similar value (5.14:1) was obtained with 2'-deoxyadenosine, but adenosine was more hydrophilic, with a value of 13.1:1.

We found evidence for neither phosphorylation (Table I) nor deamination of 5'-deoxyadenosine in cell-free extracts of L1210 cells. Chromatographic examination of cell extracts failed to show presence of any radioactive nucleotides follow-

**Phosphorylation of nucleosides by L1210 cells**

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Kinase activity nmol/mg protein/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine</td>
<td>14.6</td>
</tr>
<tr>
<td>Deoxyctidine</td>
<td>9.3</td>
</tr>
<tr>
<td>Adenosine</td>
<td>7.0</td>
</tr>
<tr>
<td>2'-Deoxyadenosine</td>
<td>5.1</td>
</tr>
<tr>
<td>5'-Deoxyadenosine</td>
<td>&lt;0.15</td>
</tr>
</tbody>
</table>

*Inhibition of Accumulation* — We tested KCN, persantin, HgCl₂/NaI, and photo-activated deuteroporphyrin IX. The latter was found (25) to cause a substantial inhibition of the accumulation of nucleosides by L1210 cells (see "Discussion").
Temperature (°C)

![Graph](image1)

**Fig. 1.** Kinetics of uptake of 5'-deoxyadenosine (■) and of adenosine (○) as a function of incubation temperature. Initial rates were measured as described in the text.

![Graph](image2)

**Fig. 2.** Plot of rate of uptake of 5'-deoxyadenosine as a function of the extracellular concentration, at 20°, measured during 18-s incubations. The mediated (M) and diffusion (D) components of transport were calculated as described in Ref. 20.

The data of Table II were obtained using 10 μM nucleoside for 18 s at 20°. The solution of HgCl₂/NaI (in isotonic NaCl) essentially abolished accumulation of adenosine, 2'-deoxyadenosine and 5'-deoxyadenosine. The porphyrin and persantin were less effective inhibitors of uptake of the latter two agents, perhaps reflecting the greater diffusion component of their uptake. In other studies, we found that a 5 to 10 mM level of KCN failed to affect accumulation of 5'-deoxyadenosine at any nucleoside level tested.

**Competition Studies**—Addition of a 250-fold excess of non-

**Fig. 3.** A, kinetics of 5'-deoxyadenosine transport, calculated from the mediated component of transport (see Fig. 2). B, rate of exodus, as a function of temperature, of 5'-deoxyadenosine from cells preloaded with 150 mM of the nucleoside. The loading incubation was carried out at 10° over a 5-min interval.

**TABLE II**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Labeled nucleoside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenosine</td>
</tr>
<tr>
<td>Persantin</td>
<td>7</td>
</tr>
<tr>
<td>HgCl₂/NaI</td>
<td>2</td>
</tr>
<tr>
<td>Porphyrin</td>
<td>8</td>
</tr>
</tbody>
</table>

**TABLE III**

<table>
<thead>
<tr>
<th>Added nucleoside</th>
<th>5'-Deoxy[³H]adenosine accumulation</th>
<th>Relative affinity of control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>16</td>
<td>2.14</td>
</tr>
<tr>
<td>2'-Deoxyadenosine</td>
<td>20</td>
<td>1.63</td>
</tr>
<tr>
<td>5'-Deoxyadenosine</td>
<td>29</td>
<td>1.00</td>
</tr>
<tr>
<td>Thymine ribose</td>
<td>30</td>
<td>0.95</td>
</tr>
<tr>
<td>Thymidine</td>
<td>48</td>
<td>0.44</td>
</tr>
<tr>
<td>Inosine</td>
<td>34</td>
<td>0.79</td>
</tr>
<tr>
<td>2'-Deoxyinosine</td>
<td>47</td>
<td>0.46</td>
</tr>
<tr>
<td>Guanosine</td>
<td>41</td>
<td>0.59</td>
</tr>
<tr>
<td>2'-Deoxyguanosine</td>
<td>51</td>
<td>0.39</td>
</tr>
<tr>
<td>Uridine</td>
<td>66</td>
<td>0.21</td>
</tr>
<tr>
<td>2'-Deoxyuridine</td>
<td>51</td>
<td>0.39</td>
</tr>
<tr>
<td>Cytidine</td>
<td>72</td>
<td>0.16</td>
</tr>
<tr>
<td>2'-Deoxyctydine</td>
<td>61</td>
<td>0.26</td>
</tr>
</tbody>
</table>

L1210 cells were incubated for 18 s at 20° with 10 μM persantin or 2 mM HgCl₂ + 1.2 mM NaI (in isotonic NaCl) + 10 μM labeled nucleoside; the cells were then collected by centrifugation, and accumulation of labeled substrate measured. Cells were incubated with 10 μg/ml of deuteroporphyrin IX in light (24), then suspended in fresh medium and incubated for 18 s at 20° with labeled nucleoside as described above. Data are shown in terms of percentage of control (untreated) values.

L1210 cells were incubated with 1 μM 5'-deoxy[³H]adenosine alone, or in the presence of 250 μM levels of other nucleosides, for 18 s at 20°. Transport was then stopped with the HgCl₂/NaI solution as described under "Methods," and accumulation of radioactive material by the cells was measured. Relative inhibition of uptake by nonradioactive compounds is shown, along with the relative affinity of competing nucleosides for transport calculated as described in Ref. 27.
radioactive nucleosides inhibited accumulation of labeled 5'-deoxyadenosine (Table III). The most potent inhibitors were adenosine, 2'-deoxyadenosine, nonradioactive 5'-deoxyadenosine, thymine riboside, inosine, and guanosine, in that order. Except for uridine and cytidine, we found the ribose derivatives to be more effective inhibitors of 5'-deoxyadenosine transport than were the 2'-deoxyribose derivatives.

Exodus of 5'-deoxyadenosine—Cells were preloaded with 150 μM of labeled compound by a 10-min incubation at 0°C, and then suspended in fresh medium at 0°C, 15°C, or 37°C. The rate of loss of label from the cells was measured as described under "Methods." The data show a substantial temperature dependence of nucleoside exodus, Fig. 3B.

DISCUSSION

This study described studies of transport of a radioactive nonmetabolized nucleoside which is not affected by the properties of intracellular nucleoside-trapping reactions, e.g., kinases. Previous reports had indicated that the kinetics of thymidine transport in cultured animal cells were altered when thymidine kinase activity was inhibited (8, 9). Since the levels of activity of nucleoside kinases are potent determinants of nucleoside accumulation, it is not always easy to distinguish effects of external agents on mediated transport versus kinase activity, and careful experiments need to be planned so that distinctions can be made, e.g. Refs. 3-5, 26.

Once a purified preparation of 5'-deoxyadenosine was obtained, we determined that this agent was not significantly phosphorylated by our model system, the transplantable murine leukemia L1210 cell line. In a recent report, the compound was also found to be neither phosphorylated nor deaminated by Ehrlich ascites tumor cells (28). Uptake of 5'-deoxyadenosine was found to be nonconcentrative; in the absence of an appropriate kinase, equilibration between external and internal nucleoside pools was very rapid. To slow the rate of uptake sufficiently to permit valid rate measurements over 6- to 18-s intervals, our experiments were mainly done at 20°C.

In contrast to the markedly temperature-sensitive uptake of adenosine (Fig. 1), 5'-deoxyadenosine uptake was much less affected by incubation temperature; furthermore, the rate of uptake was almost a linear function of temperature over the 0-37°C range (Q10 = 1.3). The Q10 for adenosine, over 27-37°C was approximately 3.0. These data suggest that the apparent temperature dependence of adenosine transport may be a more accurate reflection of the properties of adenosine kinase activity than of the mediated transport process.

We must compare kinetic properties of adenosine with those of 5'-deoxyadenosine in light of the higher octanol/water partition ratio of the latter which suggests (20) a correspondingly higher lipid solubility and a greater diffusion component of uptake. For the latter compound, the data of inhibitors of transport of labeled 5'-deoxyadenosine than was water partition ratio of the latter which suggests (20) a more accurate reflection of the properties of adenosine was found to be nonconcentrative; in the absence of an appropriate kinase, equilibration between external and internal nucleoside pools was very rapid. To slow the rate of uptake sufficiently to permit valid rate measurements over 6- to 18-s intervals, our experiments were mainly done at 20°C.

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