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/10⁸ 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 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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1 The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
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 \times 10^7/200 \mu l.

### Uptake Studies

**Accumulation of nucleosides** was measured using 200 \mu l of cell suspension + 5 \mu l of labeled substrate appropriately diluted with carrier after evaporation of ethanol. The temperature of experiment was specified. After a measured interval, we added 600 \mu l of a solution containing 2 \times 10^{-4} M HgCl_2, 1.2 \times 10^{-4} M NaI, and 100 \mu M NaCl which essentially stopped all movement of nucleoside across the cell membrane in either direction (17, 18). In some experiments, 600 \mu l of a 0.1 \mu M solution of persantin in 150 \mu M NaCl was substituted. The phases were then collected by centrifugation (30 s, 100 x g) and washed once in the HgCl_2/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 (15 \mu M final concentration) were used instead of the 5'-deoxyadenosine. The cells were then collected by centrifugation and suspended in fresh medium (5 \times 10^7 cells/ml) at 0°, 15°, or 37°. At measured intervals, 200-\mu l portions of this suspension were removed and pipetted into 600 \mu l of the HgCl_2/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 nucleosides 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 \mu M NaCl + 10 \mu M phosphate buffer, pH 7, was determined. The phases were equilibrated at room temperature, and 500 \mu l of each phase was removed and mixed to the aqueous (lower) layer was added 2 \mu l of a 5 \mu M solution of radioactive nucleoside containing 10^8 dpm. The phases were mixed, and 200-\mu l portions of each were removed for measurement of radioactivity. A 200-\mu l portion of the lower phase was then collected by centrifugation, with a fresh 200-\mu l 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 95% 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 \mu M HCl produced a labeled product with the same R_f as the major impurity described above.

The water:octanol partition ratios of 5'-deoxyadenosine was 4.0:2.1. 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 following incubation of intact cells and radioactive 5'-deoxyadenosine for several minutes at 37°. Nor was 5'-deoxyadenosine subject to any chromatographically detectable biotransformations during 3-min incubations with L1210 cells at 0°-15°.

Incubation of L1210 cells in medium containing 5'-deoxy[3H]adenosine at 20-30° for 10 min did result in formation of detectable amounts (10% of total radioactivity) of a new intracellular compound with an R_f = 0.9. The deaminase measurement on cell extracts suggests that this product is not 5'-deoxyadenosine; in contrast formation of 2'-deoxyadenosine during incubation of rat tumor cells with radioactive 2'-deoxyadenosine was found (23). Under conditions employed for studies to determine kinetic constants for 5'-deoxyadenosine transport, we therefore found no evidence for metabolism of the nucleoside.

### Kinetics of Nucleoside Uptake

Since both adenosine and 2'-deoxyadenosine are subject to intracellular phosphorylation and trapping, kinetics of uptake, in cultured cells, can be measured over 5- to 10-min intervals (24, 25). However, we found that uptake of 5'-deoxyadenosine was complete (distribution ratio = 0.9) within 12 s at 37°. A linear uptake rate, over 30 s was found at 20°, a temperature we therefore used to measure transport kinetics of the compound over 18-s intervals. Uptake was linear with time for at least 1 min at 15°; 5 min at 0°. In no case was concentrative uptake found. The influence of temperature on the initial uptake rate of 5'-deoxyadenosine and adenosine are shown in Fig. 1. Over the 27-37° range, we observed a Q_{10} of 3.0 for adenosine, but the rate of accumulation of the nucleoside was markedly reduced at temperatures below 20°. In contrast, a Q_{10} of 1.3 was found over the 0-37° range for 5'-deoxyadenosine.

When the external 5'-deoxyadenosine concentration was varied, the initial rate of uptake followed kinetics commonly observed when both mediated and nonmediated (diffusion) components of uptake are present (20). The data (Fig. 2) were then utilized, as described in Ref. 20, to determine both components of the transport process. We found an apparent K_m of 115 \mu M, with a V_{max} of 105 pmol/10^6 cells/min for the mediated transport process (Fig. 3A).

In similar experiments (data not shown), we obtained these values for adenosine, K_m = 5 \mu M; V_{max} = 110 pmol/10^6 cells/min; for 2'-deoxyadenosine, K_m = 65 \mu M, V_{max} = 90 pmol/10^6 cells/min. These data are in agreement with literature reports (24, 25).

**Inhibition of Accumulation** — We tested KCN, persantin, HgCl_2/NaI, and photo-activated deuterophosphoryl IX. The latter was found (25) to cause a substantial inhibition of the accumulation of nucleosides by L1210 cells (see "Discussion").

### Table I

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Kinase activity (nmol/mm protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine</td>
<td>14.6</td>
</tr>
<tr>
<td>Deoxycytidine</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>

2 TLC (cellulose) with Solvent R.
5'-Deoxyadenosine Transport

Temperature ('C)

FIG. 1. Kinetics of uptake of 5'-deoxyadenosine (A) and of adenosine (B) as a function of incubation temperature. Initial rates were measured as described in the text.

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-**

**TABLE II**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Adenosine</th>
<th>2'-Deoxyadenosine</th>
<th>5'-Deoxyadenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persantin</td>
<td>7</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>HgCl₂/NaI</td>
<td>2</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Porphyrin</td>
<td>8</td>
<td>11</td>
<td>18</td>
</tr>
</tbody>
</table>

**TABLE III**

<table>
<thead>
<tr>
<th>Added nucleoside</th>
<th>5'-Deoxy[¹³H]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'-Deoxycytidine</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 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.

Inhibition of 5'-deoxyadenosine transport by heterologous nucleosides

L1210 cells were incubated with 1 μM 5'-deoxy[¹³H]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, and 2'-deoxyribose phosphates. In another report (24), Plagemann found inosine, uridine, and thymidine to be the most potent nucleoside inhibitors of adenosine transport, while 2'-deoxyadenosine uptake was most effectively inhibited by deoxyinosine, adenosine, and guanosine (25). These data suggest that 5'-deoxyadenosine may enter the cell via several access systems, mainly that normally utilized by adenosine.

We found that uptake of 5'-deoxyadenosine was not inhibited by KCN, and was inhibited by persantin and a membrane-disruptive porphyrin (26), but to a lesser extent than either 2'-deoxyadenosine and adenosine. The data of Table II suggest that these two inhibitors are most effective against adenosine, a compound with only a minor capacity to diffuse into cells at low external concentrations. The HgCl2/Nal solution blocked transport of all three nucleosides, including exodus of 5'-deoxyadenosine.

We propose 5'-deoxyadenosine as a nonmetabolized nucleoside analog for the study of nucleoside transport, and the effect thereon of external agents. The only drawbacks appear to be the need for rapid sampling times or reduced temperature of incubation and the somewhat greater diffusion component of transport than is found for the ribonucleosides, e.g., adenosine.

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REFERENCES

Transport of a nonphosphorylated nucleoside, 5'-deoxyadenosine, by murine leukemia L1210 cells.

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