Function of 5’-Nucleotidase in the Uptake of Adenosine from AMP by Human Lymphocytes*

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The function of 5’-nucleotidase in nucleoside uptake from AMP was investigated in human lymphocytes by comparing the transport in cells containing this enzyme (5’N+) with that in cells deficient in the activity (5’N-). The rate of adenosine and P, uptake from AMP was 3.9-fold greater in the 5’N+ than in the 5’N- lymphocytes. There was no difference in transport between these cells when incubated with adenosine or P, These results indicate that phosphorylytic cleavage of AMP by 5’-nucleotidase is necessary for the uptake of the nucleotide and P, moieties by the human lymphocyte.

The enzyme 5’-nucleotidase (EC 3.1.3.5) which catalyzes the phosphorylytic cleavage of 5’-nucleotides, is present in many tissues and is generally associated with the plasma membrane (1, 2). This activity was shown by DePierre and Karnovsky (1, 2) to be localized to the external aspect of guinea pig granulocytes. The enzyme’s function in vivo is uncertain, but a possible role for it in purine transport has been suggested. This involves the dephosphorylation of 5’-nucleotides, to which cells are generally impermeable, to the readily transported nucleosides (2).

Previous studies from this laboratory have demonstrated that 5’-nucleotidase is present in lymphocytes from normal subjects. This activity is decreased or undetectable in lymphocytes from most patients with chronic lymphocytic leukemia (3, 4). The altered 5’-nucleotidase level has not been shown to be associated with any other metabolic differences. The availability of these homogeneous cell populations, designated as 5’N+ and 5’N-, respectively, provided an opportunity to determine the role of this enzyme in the degradation of nucleotides and uptake of nucleosides.

MATERIALS AND METHODS

[2-3H]Adenosine, 16 Ci/mmol, was obtained from Schwarz/Mann and New England Nuclear. The following were purchased from Worthington: adenosine (3P) monophosphate, 14.9 Ci/mmol; [2-3H]adenosine 5’-triphosphate, 26.0 Ci/mmol; [8-3H]adenosine 5’-monophosphate, 14.9 Ci/mmol; and Aquasol liquid scintillation mixture. P,Nitrobenzylthioinosine was a generous gift from Dr. A. R. P. Paterson (5). Bacterial alkaline phosphatase (0.75 unit) for 1 hour to dephosphorylate the nucleotides and further characterize nucleosides. These were identified by paper chromatography in water, as well as in the NH4OH/isobutyrilic acid.

RESULTS

The rate of adenosine uptake from [3H]AMP was 3.9 times greater in 5’N+ than in 5’N- lymphocytes (Fig. 1A, Table I). The 5’N+ lymphocytes transported an average of 5.4 ± 0.9 pmol, while the 5’N- cells transported 1.4 ± 0.2 pmol of [3H]adenosine/30 min/106 cells (p < 0.001). The uptake of 3P, from [3P]AMP was determined in the 5’N+ and 5’N- cells (Fig. 2A, Table I). Uptake of [3P]-AMP by 5’N+ lymphocytes was 3.9 times greater than by 5’N- lymphocytes (p < 0.02). The rate of [3H]adenosine uptake from [3H]AMP was identical in cells from normal subjects and 5’N+ cells from patients with chronic lymphocytic leukemia, indicating that the differences between 5’N+ and 5’N- cells reflect the presence or absence of 5’-nucleotidase activity and not whether the cells came from a normal or leukemic donor.

The diminished uptake of the adenosine and P, moieties of AMP by 5’N- cells could reflect either a failure to cleave the AMP to the nucleoside + P, or alternatively a defect in the transport of these compounds. To investigate these possibili-

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ties, the transport of $[^{3}H]$adenosine and $[^{32}P]$P$_{i}$ was compared in these populations. In the 5'N+ cells the uptake of $[^{3}H]$adenosine was 9.9 ± 1.1 pmol/30 min/10^6 cells, while in the 5'N- cells 11.7 ± 1.7 pmol/30 min/10^6 cells was taken up (Fig. 1B, Table I). There was no significant difference in uptake of $[^{3}H]$adenosine between 5'N+ and 5'N- cells. When $[^{32}P]$P$_{i}$ was incubated with 5'N+ and 5'N- lymphocytes, identical rates of uptake were also observed for this compound (Fig. 2B).

The effect of dilution with unlabeled adenosine or P$_{i}$ on the uptake of label from AMP is shown in Fig. 3A. A 10-fold molar excess of unlabeled adenosine diluted out the counts taken up from $[^{3}H]$AMP but not from $[^{32}P]$AMP, consistent with the interpretation that AMP is split by 5'-nucleotidase before rather than after uptake by the cell. The addition of a 10-fold molar excess of unlabeled P$_{i}$ (not shown) had no effect on the uptake. Further increases in the concentration of P$_{i}$ up to 0.1 M (Fig. 3B) also had no effect on the uptake of counts. The control experiments showing the effect of unlabeled adenosine and P$_{i}$ on the uptake of $[^{3}H]$adenosine are shown in Fig. 3C.

The results were similar to those for the uptake from AMP. The inability to dilute out the counts of P$_{i}$ taken up is consistent with the suggestion that a much greater quantity of P$_{i}$ than of adenosine is accumulated by the human lymphocyte. This

**Table I**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>$[^{3}H]$AMP</th>
<th>$[^{32}P]$AMP</th>
<th>$[^{3}H]$Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'N+ (12)</td>
<td>6.4 ± 0.9</td>
<td>6.1 ± 1.1</td>
<td>0.9 ± 1.1</td>
</tr>
<tr>
<td>5'N- (10)</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.6</td>
<td>11.7 ± 1.7</td>
</tr>
</tbody>
</table>

Adenosine and P$_{i}$ uptake by human lymphocytes. The 5'N+ lymphocytes were isolated from 10 normal subjects and 12 patients with chronic lymphocytic leukemia. The 5'N- lymphocytes have an average specific activity of 0.28 (0.07 to 0.63) pmol/hour/mg. The 5'N+ cells have a specific activity of 0.02 (0.01 to 0.05) pmol/hour/mg. Transport was determined from the linear portion of the uptake curve under conditions given under “Materials and Methods.” Since identical results were obtained with the 5'N+ cells from normal subjects or patients with chronic lymphocytic leukemia, these were not subdivided further. Numbers in parentheses indicate number of subjects.

FIG. 1. Uptake of adenosine by human lymphocytes. Cells were incubated as described under “Materials and Methods.” The 5'-nucleotidase levels in 5'N+ (△-△) and 5'N- (○-○) cells were, respectively, 0.43 and 0.03 pmol/hour/mg. A, uptake of adenosine from $[^{3}H]$AMP. The nucleotide was added to the incubation mixture at a 1 μM concentration. B, uptake of adenosine from the medium. The nucleoside concentration was 1 μM.

FIG. 2. Uptake of $[^{32}P]$P$_{i}$ by human lymphocytes. Cells were incubated as described under “Materials and Methods.” The 5'-nucleotidase levels in 5'N+ (△-△) and 5'N- (○-○) cells were, respectively, 0.43 and 0.01 μmol/hour/mg. A, uptake of $[^{32}P]$P$_{i}$ from $[^{32}P]$AMP. The nucleotide concentration was 1 μM. B, uptake of $[^{32}P]$P$_{i}$ from the medium. The concentration of P$_{i}$ in the medium was 10 mM.

FIG. 3. Effect of dilution on the uptake of $[^{3}H]$adenosine and $[^{32}P]$P$_{i}$ by human lymphocytes. Cells were incubated as described under “Materials and Methods.” A, uptake of $[^{3}H]$adenosine from $[^{3}H]$AMP. The nucleotide was added to the incubation mixture at a 1.8 μM concentration (△-△). Unlabeled adenosine was added to the incubation mixture at a 26 μM concentration (△-△) and P$_{i}$ at a 0.1 M concentration (○-○). B, uptake of $[^{32}P]$P$_{i}$ from $[^{32}P]$AMP. The nucleotide concentration was 2.5 μM (△-△). Unlabeled adenosine was added to the incubation mixture at 26 μM concentration (△-△) and P$_{i}$ at 0.1 M concentration (○-○). C, uptake of $[^{3}H]$adenosine. The nucleotide concentration was 2.6 μM (△-△). Unlabeled adenosine was added to the incubation mixture at 26 μM concentration (△-△) and P$_{i}$ at 0.1 M concentration (○-○).
interpretation is supported by the data in Fig. 2B which indicate that the uptake of P, (0.4 nmol/30 min) is at least 9-fold greater than the \( V_{\text{max}} \) for adenosine (see below). The \( V_{\text{max}} \) transport system was not characterized in this cell type. The above experiments were performed only with 5'N+ cells and could not be performed on 5'N- cells because of the failure by the latter cell to transport labeled adenosine or P, from AMP.

The adenosine transport system was characterized further in several ways (Fig. 4, Table II). The \( K_m \) for adenosine transport determined by the double reciprocal plot was 1.2 \( \mu M \) (average of four experiments), while the \( V_{\text{max}} \) was 44 pmol/30 min/10^6 cells. This \( K_m \) was 8.3-fold less than that reported in rabbit polymorphonuclear leukocytes (8). The uptake decreased by 74% when the mixture was incubated at 4°C. Adenosine uptake decreased only slightly when the cells were incubated with iodoacetate and NaCN. Deoxyglucose and NaF had no effect.

As has been previously reported for rabbit polymorphonuclear leukocytes (8, 9), borate ion inhibits adenosine transport presumably by complexing with the ribose moiety of the nucleoside.

In order to determine whether adenosine transport was occurring by simple or facilitated diffusion, the uptake of adenosine was determined in the presence or absence of p-nitrobenzylthioinosine, a compound which is considered to be a specific inhibitor of the facilitated transport system for adenosine in erythrocytes (5). In the presence of 1 \( \mu M \) p-nitrobenzylthioinosine (Fig. 5), the uptake of adenosine, added as the nucleoside, or liberated from AMP, was inhibited. An unexpected observation was the diminished uptake of \(^{32}P_1\) from \(^{32}P\)-AMP or from \(^{32}P_1\), added to the medium (Fig. 6) in the presence of the inhibitor. The inhibitory effect of p-nitrobenzylthioinosine on phosphate transport would suggest either that this compound is not as specific an inhibitor as heretofore considered or that the adenosine and phosphate transport systems in the lymphocyte can be functionally or morphologically linked.

Further evidence that 5'N- cells were capable of normal transport of nucleosides was obtained by performing the incubation in the presence of 10% autologous serum as a source

![Fig. 4. Double reciprocal plot of rate of adenosine uptake against the concentration of adenosine in the incubation medium.](image)

**Table II**

Effect of temperature and inhibitors in adenosine transport

Transport was determined from the linear portion of the uptake curve under conditions given under "Materials and Methods."

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>( \text{Pmol} ) [^{3}H\text{adenosine/30 min/10}^6 \text{ cells} )</th>
<th>% Decrease in transport</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine at 37°C</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine at 4°C</td>
<td>5</td>
<td>74%</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine + 2 x 10^-4 ( M ) iodoacetate</td>
<td>7</td>
<td>30%</td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine + 10^-3 ( M ) NaCN</td>
<td>7</td>
<td>30%</td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine + 6 mm 2-deoxyglucose</td>
<td>9</td>
<td>No effect</td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine + 2 x 10^-4 ( M ) NaF</td>
<td>9</td>
<td>No effect</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1.0 ( \mu M ) Adenosine + 20 mm Borate</td>
<td>4</td>
<td>50%</td>
</tr>
</tbody>
</table>

![Fig. 5. Effect of p-nitrobenzylthioinosine on adenosine transport by human lymphocytes. The experimental conditions for incubation with \[^{3}H\text{AMP (A)}\) and \[^{3}H\text{adenosine (B)}\) are as described in Fig. 1. Cells were incubated in the absence (△—△) or in the presence (Δ—Δ) of the inhibitor.](image)

![Fig. 6. Effect of p-nitrobenzylthioinosine on \(^{32}P_1\) transport by human lymphocytes. The experimental conditions for incubation with \(^{32}P\text{AMP (A)}\) and \(^{32}P_1\) (B) are as described for Fig. 2 and in the text.](image)
of exogenous 5'-nucleotidase. Under these assay conditions uptake of [\(^{3}H\)]adenosine from [\(^{3}H\)]AMP or [\(^{32}P\)]AMP was identical in both cell types.

Similar intermediates were observed following uptake of adenosine in the 5'N+ and 5'N- cells. Following a 15-min incubation with [\(^{3}H\)]adenosine, approximately 70% of the radioactivity was isographic with ATP and almost all of the remaining radioactivity with ADP. After 60 min of incubation, in addition to the radioactivity associated with the adenosine nucleotides, approximately 15% of the radioactivity was isographic with GMP. A similar distribution was found for adenosine taken up from [\(^{3}H\)]AMP, suggesting that rephosphorylation of the nucleoside products was occurring.

**DISCUSSION**

Leukocytes do not carry out de novo synthesis of purines (10). As such, these cells are dependent, in vivo or in vitro, on the availability of purine nucleotides or their nucleoside moiety from the environment. Since leukocytes are generally impermeable to nucleotides, the dephosphorylation by 5'-nucleotidase would present the cell with a compound that is readily transported and metabolized. DePierre and Karnovsky have elegantly demonstrated that guinea pig polymorphonuclear leukocyte 5'-nucleotidase is ideally suited for this function, being an ecto-enzyme (1), i.e. an enzyme whose active site faces the external medium. The human lymphocyte 5'-nucleotidase has a similar localization (4).

The experiments reported above clearly demonstrate the dependence of adenosine uptake from AMP on the presence of 5'-nucleotidase. There was a striking difference in the uptake of [\(^{3}H\)]adenosine from [\(^{3}H\)]AMP between 5'N+ and 5'N- lymphocytes, while identical uptake of [\(^{3}H\)]adenosine was observed when cells were incubated with this nucleoside. The transport differences were dependent on the presence of the 5'-nucleotidase, and not on whether cells came from a normal or leukemic donor. The subsequent metabolism of adenosine was similar in the 5'N+ and 5'N- cells. Further evidence for this function of 5'-nucleotidase is offered by the finding that the addition of 5'-nucleotidase to the incubation medium abolished the difference between the 5'N+ and 5'N- cells, indicating that 5'N- lymphocytes were normal with respect to the transport of the nucleoside and P, A similar role has recently been demonstrated for 5'-nucleotidase in bacteria using a mutant strain deficient in this enzyme (11).

Several differences occur in the mechanism by which purines and purine derivatives are transported and metabolized in bacteria and mammalian cells. These include the ability of taking up free bases by bacteria which is not found in mammalian cells, and the pathway by which bacteria degrade nucleotides to the free base and then rephosphorylate the free base to a nucleotide. The data presented above suggest that the function of 5'-nucleotidase is one area of similarity between the way prokaryotes and eukaryotes handle the nucleotide prior to transport into the cell.

It remains uncertain whether the absence of this activity presents a physiological disadvantage to the cell in vivo since phosphatases in the surrounding plasma environment would cleave the nucleotides and make the nucleoside available to the cell.

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