Alternative Pathways of Deoxyadenosine and Adenosine Metabolism

Floyd F. Snyder and J. Frank Henderson

From the Cancer Research Unit (McCachern Laboratory) and Department of Biochemistry, University of Alberta, Edmonton T6G 2E1, Alberta, Canada

SUMMARY

The relative rates of phosphorylation, deamination, and cleavage of deoxyadenosine and adenosine were examined in Ehrlich ascites tumor cells; mouse, human, and sheep erythrocytes; and mouse brain, heart, kidney, and liver. Cleavage of deoxyadenosine was measured by the formation of adenine ribonucleotides and phosphorylation by the formation of adenine deoxyribonucleotides; deamination of deoxyadenosine was inhibited by coformycin. All of the tissues examined exhibited a low but measurable ability to cleave the glycosidic bond of deoxyadenosine. The deoxyadenosine kinase to cleavage ratio was greatest, 3.5 to 6, in erythrocytes, 2.1 for Ehrlich ascites tumor cells, and near unity for mouse tissues. In the absence of the adenosine deaminase inhibitor, deamination was the major route of deoxyadenosine metabolism in mouse brain, heart, kidney, and liver and in mouse and human erythrocytes, whereas phosphorylation and deamination were nearly equivalent in sheep erythrocytes. At low substrate concentrations (less than 150 μM) more adenosine was phosphorylated than deaminated in Ehrlich ascites tumor cells.

Deoxyadenosine and adenosine can be metabolized in animal cells both by phosphorylation and by deamination. The relative rates of phosphorylation and deamination of deoxyadenosine are known to vary from one animal cell type to another, but their relationship has not been studied systematically. In Ehrlich ascites tumor cells incubated with deoxyadenosine, for example, large amounts of dADP and dATP were found to accumulate (1, 2). Withdrawal of the deoxyadenosine, however, resulted in rapid degradation of dATP primarily to hypoxanthine, although small amounts of deoxyadenosine and deoxyinosine were also observed (1). In contrast, radioactivity from deoxyadenosine was incorporated primarily into RNA rather than into DNA in human lymphocytes (3), suggesting that deamination followed by cleavage of the glycosidic bond of deoxyinosine was of major importance.

Recently Zimmerman et al. (4) have shown that purine nucleoside phosphorylase can convert adenine to adenosine, and this observation raises the possibility that adenine nucleosides can also be cleaved to adenine in intact animal cells. This possibility is explored in the present study, which also determines the relative rates of deoxyadenosine metabolism via phosphorylation, deamination, and cleavage in several different animal cells and tissues.

METHODS AND MATERIALS

Chemicals—Radioactive bases and nucleosides were purchased from New England Nuclear Corp.: [8-14C]deoxyadenosine, 39 mCi per mmole; [8-14C]inosine, 32 mCi per mmole; [8-14C]ad-
dene, 52.6 mCi per mmole; [8-14C]guanine, 52 mCi per mmole; and from Schwarz BioResearch, Inc.: [8-14C]adenosine, 47 mCi per mmole; [8-14C]hypoxanthine, 53.7 mCi per mmole. Coformycin was generously provided by Professor H. Umezawa and Dr. M. Ifori, Institute of Microbial Chemistry, Tokyo, and 2,6-dichloro-9-(tetrahydroprop-2-yl)-9H-purine was provided by the Cancer Chemotherapy National Service Center, Bethesda, Md.

14C]Deoxyadenosine was purified prior to each experiment by chromato
graphing 100 to 200 nl on Eastman Kodak unsubstituted cellulose thin layer sheets in 1-butanol-methanol-water-ammonia (60:20:20:1); sheets were developed twice. This procedure separated deoxyadenosine from adenine, adenosine, hypoxanthine, and inosine. The deoxyadenosine spot was scraped off the chromatogram and extracted with four 0.5-ml aliquots of water. The combined aqueous extracts were evaporated to dryness in vacuo over NaOH flakes and resuspended in the original sample volume. Only deoxyadenosine preparations free from radioactive adenine, adenosine, inosine, and hypoxan-
thine were used for the experiments reported.

Adenosine deaminase in Ehrlich ascites tumor cells has been shown to be inhibited by more than 99% by 1 μg per ml coformycin (5). In this study, 5 μg per ml of coformycin were used in experiments employing mouse, sheep, and human erythrocytes and Ehrlich ascites tumor cells, whereas 10 μg per ml were used when preparations of mouse brain, kidney, heart, and lung were studied. Control experiments showed that in all cases the deamination of 14C]deoxyadenosine was virtually completely inhibited by these concentrations of coformycin.

Tissue Preparation and Incubation Conditions—Ehrlich ascites tumor cells were collected and diluted in Fischer’s medium containing 25 mM phosphate buffer, pH 7.4, without bicarbonate, to make a 2.5% cell suspension. The tumor cell suspensions...
(80 µl) with or without coformycin were incubated in small plastic tubes (10 × 75 mm, Falcon Plastics) for 20 min at 37°C with shaking. Radioactive nucleoside and Fischer's medium were then added to a final volume of 100 µl, and the incubation continued. Experiments were terminated by addition of 5 µl of 4.2 M cold perchloric acid to each tube, and the cell extract was neutralized by the addition of 5 µl of 4.42 M KOH. Samples were chilled, centrifuged, and 10 µl of the supernatant were chromatographed.

Mouse, sheep, and human erythrocytes were collected in modified Fischer's medium containing heparin, then washed and diluted to 2.5% suspension in modified Fischer's medium, and incubated as above with radioactive precursors in the presence or absence of coformycin with 100% O₂ in the gas phase.

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Fig. 1. Metabolism of [³⁴C]deoxyadenosine in Ehrlich ascites tumor cells. Cells were incubated with [³⁴C]deoxyadenosine for 20 min and radioactivity in the products of deoxyadenosine kinase, dAMP + dADP + dATP (○), and deoxyadenosine cleavage, AMP + ADP + ATP (△), was measured. The concentration of coformycin was 5 μg per ml.

Fig. 2. Phosphorylation of [¹⁴C]deoxyadenosine in erythrocytes. Human (○), mouse (△), and sheep (□) erythrocytes were incubated with [¹⁴C]deoxyadenosine for 30 min after which radioactivity in dAMP + dADP + dATP was measured. Human and mouse erythrocytes were incubated with 5 μg per ml of coformycin and sheep erythrocytes in the absence of coformycin.

Fig. 3. Cleavage of [¹⁴C]deoxyadenosine in erythrocytes. Human (○), mouse (△), and sheep (□) erythrocytes were incubated with [¹⁴C]deoxyadenosine for 30 min after which radioactivity in AMP + ADP + ATP was measured. Human and mouse erythrocytes were incubated with 5 μg per ml of coformycin and sheep erythrocytes in the absence of coformycin.

Fig. 4. Metabolism of [¹⁴C]deoxyadenosine in mouse tissues. Tissues were incubated with [¹⁴C]deoxyadenosine (20 μM) for 20 min and radioactivity in products of deoxyadenosine kinase, dAMP + dADP + dATP (open bar) and deoxyadenosine cleavage, AMP + ADP + ATP (hatched bar) was measured. The concentration of coformycin was 10 μg per ml.

Chopped prisms of mouse kidney, liver, and brain and slices of mouse heart were incubated with 20 μM [¹⁴C]deoxyadenosine in the presence of coformycin (10 μg per ml) for 20 min. Under these conditions there was no detectable deamination of deoxyadenosine, judged by the lack of accumulation of radioactivity in deoxyinosine and hypoxanthine. All of these tissues had sufficient adenine phosphoribosyltransferase under the conditions of these experiments for the assay of deoxyadenosine cleavage. (9) Fig. 4 shows that of the four mouse tissues studied, deoxyadenosine kinase and cleaving activities were highest in kidney; brain and liver had comparable activities which were lower than kidney, and these activities in heart were close to the limit of sensitivity of the methods used. A comparison of deoxyadenosine kinase and cleaving activities in all the cells and tissues examined is given in Table 1.

The metabolism of deoxyadenosine was also measured in the absence of coformycin to determine the relative rates of phosphorylation and deamination of this nucleoside. Incubation of mouse liver, kidney, heart, or brain for 20 min in the absence of coformycin resulted in the deamination of more than 90% of the 20 μM [¹⁴C]deoxyadenosine present as measured by radioactivity in deoxyinosine and hypoxanthine; ribonucleotides accounted for a further 1 to 5% of the total radioactivity. In these tissues cleavage cannot be measured in the presence of such extensive deaminase activity because deoxyinosine can be converted to adenine ribonucleotides via hypoxanthine and inosinate.

1 P. C. L. Wong and J. F. Henderson, unpublished results.
In contrast, there was essentially no synthesis of ATP or GTP from radioactive hypoxanthine in both human and sheep erythrocytes, and deoxyadenosine cleaving activity can hence be measured in the absence of coformycin. (In both types of cells, radioactivity in both ATP and GTP following incubation with [14C]hypoxanthine was approximately 2% of the radioactivity in inosinate.) Deoxyadenosine deaminase, kinase, and cleaving activities in human and sheep erythrocytes incubated without coformycin are given in Table II. In human erythrocytes, deamination of deoxyadenosine was some 75- to 180-fold greater than phosphorylation, whereas in sheep erythrocytes deamination and phosphorylation of deoxyadenosine were roughly equivalent. The ratio of cleaving activity to those of the other processes was several-fold higher in sheep erythrocytes than in human erythrocytes, although total cleaving activity was higher in human erythrocytes.

**Table I**

**Relative activities of deoxyadenosine phosphorylation and cleavage**

Cells were incubated with [14C]deoxyadenosine and the ratio of radioactivity in the products of deoxyadenosine phosphorylation, dAMP + dADP + dATP, and deoxyadenosine cleavage, AMP + ADP + ATP, was determined. Human and mouse erythrocytes, and Ehrlich ascites tumor cells, 2% (v/v) suspensions were incubated with 5 μg per ml of coformycin; sheep erythrocytes, 2% (v/v) suspension, were incubated without coformycin; and chopped mouse brain, kidney, and liver and sliced mouse heart were incubated with 10 μg per ml of coformycin.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Deoxyadenosine concentration μg/ml</th>
<th>Ratio of phosphorylation to cleavage</th>
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<tbody>
<tr>
<td>Human erythrocytes</td>
<td>20-42</td>
<td>6.0</td>
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<tr>
<td>Mouse erythrocytes</td>
<td>16-50</td>
<td>3.6</td>
</tr>
<tr>
<td>Sheep erythrocytes</td>
<td>16-33</td>
<td>3.4</td>
</tr>
<tr>
<td>Ehrlich ascites tumor cells</td>
<td>10-56</td>
<td>2.1</td>
</tr>
<tr>
<td>Mouse brain</td>
<td>20</td>
<td>1.2</td>
</tr>
<tr>
<td>Mouse kidney</td>
<td>20</td>
<td>1.1</td>
</tr>
<tr>
<td>Mouse heart</td>
<td>20</td>
<td>1.0</td>
</tr>
<tr>
<td>Mouse liver</td>
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</tr>
</tbody>
</table>

**Table II**

**Comparison of deoxyadenosine deaminase, kinase, and cleavage activities in human and sheep erythrocytes**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Deoxyadenosine</th>
<th>Deaminase</th>
<th>Kinase</th>
<th>Cleavage</th>
<th>Relative activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human erythrocytes</td>
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<td>10.4</td>
<td>7.9</td>
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<tr>
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<td>41.6</td>
<td>36.1</td>
<td>13.4</td>
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<td>62.0</td>
<td>49.3</td>
<td>20.4</td>
<td>98.1</td>
<td>1.3</td>
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<tr>
<td>Sheep erythrocytes</td>
<td>16.7</td>
<td>14.8</td>
<td>13.2</td>
<td>46.5</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
<td>24.3</td>
<td>26.1</td>
<td>42.0</td>
<td>45.2</td>
</tr>
</tbody>
</table>

A comparison of deoxyadenosine kinase and deaminase activities in intact Ehrlich ascites tumor cells is shown in Fig. 6. At low substrate concentrations several times more deoxyadenosine is phos-
phosphorylated than deaminated, as would be predicted from the Michaelis constants of adenosine for adenosine kinase, 8 to 6 × 10⁻⁶ M (11, 12), and adenosine deaminase, 3 × 10⁻³ M (13). At high concentrations of adenosine (greater than 150 μM), deamination of adenosine was greater than phosphorylation. Phosphorylation of adenosine by Ehrlich ascites tumor cells was 5-fold greater than that of deoxyadenosine at concentrations of 16 μM.

In sheep erythrocytes adenosine deaminase activity is shown in Fig. 6 to be greater than adenosine kinase activity over the whole range of adenosine concentrations studied. Deamination of deoxyadenosine was 4-fold lower than deamination of equal concentrations of adenosine in these cells. Adenosine and deoxyadenosine kinase activities were nearly equivalent, but were low in comparison to other cells. To determine whether the low activities of enzymes of adenosine and deoxyadenosine metabolism in sheep erythrocytes were related to limited uptake of nucleoside, these cells were incubated with [¹⁴C]inosine, and nucleoside phosphorylase activity measured by determination of the accumulation of radioactive hypoxanthine (Fig. 7). Inosine phosphorylase activity in sheep erythrocytes was found to be approximately 35-fold greater than adenosine deaminase activity.

In view of the low levels of adenosine kinase and deaminase activities observed in sheep erythrocytes, the capacity of these cells to synthesize nucleotides from purine bases was also examined. Nucleotide synthesis from 100 μM [¹⁴C]adenine, [¹⁴C]hypoxanthine, and [¹⁴C]guanine was 156, 55, and 6 nmoles per g per 60 min, respectively. For comparison, in human erythrocytes nucleotide synthesis from 125 μM [¹⁴C]adenine and 100 μM [¹⁴C]hypoxanthine was 355 and 205 nmoles per g per 60 min, respectively.

In view of the evidence presented above for the formation of adenine from deoxyadenosine, several attempts were made to determine whether adenine was also formed from adenosine in Ehrlich ascites tumor cells. Cells containing ATP labeled with [¹⁴C]hypoxanthine were incubated with 5.5 mM 2-deoxyglucose to cause ATP breakdown, 1 μg per ml of coformycin to inhibit adenosine deaminase, and 5 mM 2,6-dichloro-9-(tetrahydropyrimidin-2-yl)-9H-purine, which inhibited nucleotide synthesis from adenine by 83%. A small amount of adenine (5 nmoles per g) accumulated after 50 min, suggesting some cleavage of adenosine to adenine. Because of the incomplete inhibition of adenosine phosphorylase transferase activity, however, the exact extent of this process cannot be determined.

**DISCUSSION**

Alternative routes of deoxyadenosine metabolism have been examined in Ehrlich ascites tumor cells; mouse, human, and sheep erythrocytes; and mouse brain, heart, kidney, and liver. All of these tissues exhibited a low but measurable ability to cleave the glycosidic bond of deoxyadenosine. Although most previous studies of purine nucleoside phosphorylase from a variety of sources indicated that adenosine and deoxyadenosine were not substrates (14), recent experiments indicate that this enzyme from several mammalian sources can convert adenosine to adenine at low rates in the presence of ribose 1-phosphate (4). Although studies of the metabolism of generally labeled adenosine in rat erythrocytes provided no evidence for phosphorylase of adenosine, the sensitivity of the methods used was not as great as those used here.

There are but a few previous reports providing information on adenine formation in mammalian cells. Although adenine has not been detected in normal serum (15) or tissues in vivo (16, 17), 30 min of ischemia (18), leading to the degradation of nucleotides, caused accumulation of adenosine and adenine in rat heart (480 and 80 nmoles per g) and brain (340 and 60 nmoles per g). Adenosine and adenine were also detectable in rabbit kidney and liver under similar conditions (17). Of the four mouse tissues examined in this study, kidney exhibited the highest deoxyadenosine cleaving activity. It is also known that small amounts of adenine, 1.4 μg per day, are excreted in human urine (18). The origin of urinary adenine is unclear, but the widespread distribution of adenine phosphorylase transferase (19-21) may account for the lack of free adenine in tissues.

Further indirect evidence for a mammalian adenine cleaving activity has come from studies of H. Ep. No. 2/MEMPR cells, which have adenine phosphorylase transferase but not adenosine kinase activities, and which are resistant to 6-methylmercapto-purine ribonucleoside. Contrary to expectation, they were not cross-resistant to 2-fluoroadenosine, suggesting that 2 fluoro adenosine was converted to nucleotide by the sequential action of purine nucleoside phosphorylase and adenine phosphorylase transferase (22).

There appears to be some variation in the substrate specificity of adenosine kinases from different tissues. The data of Wong and Henderson (9) indicate that in chopped mouse brain, phosphorylation of adenosine (9) was 70-fold greater than phosphorylation of deoxyadenosine (Fig. 4) at 20 μM nucleoside concentration. Sheep erythrocytes, however, phosphorylated nearly equivalent amounts of deoxyadenosine and adenosine. Deoxyadenosine is phosphorylated by mammalian adenosine kinases from Ehrlich ascites tumor cells and rabbit liver (11), but by adenosine kinase purified from H. Ep. No. 2 cells (23) or yeast (24, 25). Deoxyadenosine kinase purified from calf thymus used adenosine and guanosine at much lower rates than deoxyadenosine and deoxyguanosine (26). There is indirect evidence that a single enzyme is responsible for the conversion of both nucleosides to nucleoside monophosphates in the Ehrlich ascites tumor cells used in this work; adenine was phosphorylated approximately 10-fold more rapidly than deoxyadenosine (5).

A single enzyme is believed to be responsible for the deamination of deoxyadenosine and adenosine in mammalian cells (27-29). Because the inosine and deoxynosine produced by deamination are substrates of purine nucleoside phosphorylase (30), the three potential routes of deoxyadenosine metabolism, phosphorylation, deamination, and cleavage, are not ordinarily distinguished in studies of intact cells since AMP may ultimately be formed via all of them.

In the absence of coformycin, deamination was the major route of deoxyadenosine metabolism in mouse brain, heart, kidney, and liver and in mouse and human erythrocytes, whereas phosphorylation and deamination were nearly equivalent in sheep erythrocytes. Adenosine deaminase activities were nearly equal in sheep erythrocytes and Ehrlich ascites tumor cells at a concentration of 16 μM adenine, but adenine kinase activity in Ehrlich ascites tumor cells was 50 times higher than in sheep erythrocytes. Because human and sheep erythrocytes are unable to synthesize adenine nucleotides from hypoxanthine, deamination of adenosine therefore produces a reduction of adenine compounds in these cells. In view of the rapid turnover of adenine nucleotides in erythrocytes (31-33), these cells may require a continuous supply of adenine or adenosine to maintain the adenine nucleotide pool.
Acknowledgment—We wish to thank Mr. George Zumbor for excellent technical assistance.

REFERENCES