Biochemical Pharmacology, Vol. 27, pp. 2163–2169

0006-2952/78/0901-2163$02.00/0

EFFECTS OF 5′-DEOXYADENOSINE ON PHOSPHORIBOSYL PYROPHOSPHATE AND PURINE NUCLEOTIDE SYNTHESIS IN EHRLICH ASCITES TUMOR CELLS *IN VITRO**

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(*Received* 6 *September* 1977; *accepted* 12 *December* 1977)

Abstract—5′-Deoxyadenosine inhibits purine ribonucleotide synthesis *de novo*, as well as synthesis from adenine, guanine and hypoxanthine in Ehrlich ascites tumor cells *in vitro*. This is due to inhibition of the synthesis of phosphoribosyl pyrophosphate, but phosphoribosyl pyrophosphate synthetase itself is not inhibited. 5′-Deoxyadenosine affects several aspects of glucose metabolism, and it is proposed that this leads, in an as yet unknown way, to a decreased availability of ribose 5-phosphate for phosphoribosyl pyrophosphate synthesis.

Adenosine is toxic to many mammalian and microbial cells, but the basis (or bases) of its growth inhibitory and cytotoxic effects is not known for certain [1–4]. The study of adenosine toxicity is complicated by uncertainty regarding the relative importance for this phenomenon of adenosine itself, of nucleotides formed by the phosphorylation of adenosine, and of products of the deamination of adenosine. 5′-Deoxyadenosine, by the nature of its structure, cannot be converted to adenine 5′-nucleotides, and Bloch *et al.* [5] have found that, in addition, it is not a substrate for adenosine deaminase. 5′-Deoxyadenosine, therefore, might mimic whatever metabolic effects free adenosine may have, and hence a study of the effects of 5′-deoxyadenosine may contribute to an understanding of adenosine toxicity. This paper, therefore, reports studies of the effects of 5′-deoxyadenosine on purine nucleotide biosynthesis, formation of phosphoribosyl pyrophosphate (PP-ribose-P), and other aspects of glucose metabolism in Ehrlich ascites tumor cells *in vitro*.

MATERIALS AND METHODS

[8-^{14}C]hypoxanthine (58 mCi/m-mole) was purchased from Schwarz/Mann Corp.; [8-^{14}C]adenine (55 mCi/m-mole), [^{3}H]adenosine 3′ : 5′-monophosphate (39.8 Ci/m-mole), [8-^{14}C]adenosine (51 mCi/m-mole), [8-^{14}C]guanine (46 mCi/m-mole), and [1,2-^{14}C]glycine (100 mCi/m-mole) were purchased from New England Nuclear Corp., Boston, MA.

5′-Deoxyadenosine was purchased from P–L Biochemical Inc., Milwaukee, WI. No impurities were detected when it was chromatographed on Whatman No. 1 paper in 1-butanol-acetic acid-water (25:15:10, v/v). Other non-radioactive purines were purchased from Sigma Chemical Co., St. Louis, MO. 2′-Deoxycoformycin was obtained from Dr. G. A. LePage.

Glycerol 3-phosphate dehydrogenase (EC 1.1.1.8, 175 units/mg of protein), glucosephosphate isomerase (EC 5.3.1.9, 550 units/mg), glucose 6-phosphate dehydrogenase (EC 1.1.1.49, 345 units/mg), triosephosphate isomerase (EC 5.3.1.1, 6540 units/mg), fructose bisphosphate aldolase (EC 4.1.2.13, 9.9 units/mg) and 6-phosphofructokinase (EC 2.7.1.11, 215 units/mg) were obtained from Sigma Chemical Co.

Procedures for the maintenance and preparation of Ehrlich ascites tumor cells have been described previously [6].

Experiments involving the metabolism of radioactive purines were carried out in modified Fischer's medium (containing 25 mM phosphate and no bicarbonate) with total incubation volumes of 100 μl. Ehrlich ascites tumor cell suspensions (2%, v/v) were incubated for 20 min at 37° with 5′-deoxyadenosine. The radioactive precursor was then added and the incubations were continued for an additional 30 min. The incubations were terminated by addition of 4.2 M perchloric acid to a final concentration of 0.2 M. After 15 min on ice the extracts were neutralized with 4.2 M KOH.

Procedures for the separation and measurement of radioactivity in purine bases, ribonucleosides and ribonucleotides [6], and methods for the measurement of concentrations of PP-ribose-P [7], and of the incorporation of [^{14}C]glycine into phosphoribosyl formylglycineamide [8] have been reported previously.

Experiments in which $^{14}CO_2$ was collected were carried out with total incubation volumes of 2 ml in 25-ml Erlenmeyer flasks fitted with rubber ampule caps. Plastic wells containing fluted filter paper and 0.3 ml ethanolamine were suspended from the caps. Incubations were terminated by injecting 2 M perchloric acid, to a final concentration of 0.2 M, through the cap, after which shaking was continued for 1 hr. The wells were removed and the contents,

* This work was supported by the National Cancer Institute of Canada and by the Medical Research Council.

including the filter paper, were transferred to scintillation vials containing 4.5 ml of Bray's scintillation counting fluid [9].

Lactate concentrations in cell extracts were measured by the method of Hohorst as described [10] using prepared reagents from Boehringer Mannheim Co., St. Laurent, Quebec. The enzymatic assays for glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, and dihydroxyacetone phosphate were modifications of assays described by Bergmeyer [11, 12]. Cyclic AMP concentrations were determined on 2% cell suspensions of Ehrlich ascites tumor cells as described by Lauzon *et al.* [13]. The cells were incubated for 30 min at 37° in Krebs–Ringer high phosphate medium [6] containing 5.5 mM glucose. Incubations were terminated by transferring the cell suspensions to microcentrifuge tubes containing 0.5 ml of cold 0.6 M trichloroacetic acid and [^{3}H]cAMP to monitor recovery of cyclic AMP.

The preparation of partially purified adenine phosphoribosyltransferase (EC 2.4.2.7) from Ehrlich ascites tumor cells has been described previously [14]. Partially purified PP-ribose-P synthetase (EC 2.7.6.1) was prepared as follows. Ehrlich ascites tumor cells were collected from ICR Swiss mice 6 days after implantation and washed twice with 0.1 M NaH_2PO_4 buffer (pH 7.4) containing 1 mM reduced glutathione. The following steps were carried out at 4°. Packed cells (1.5 ml) were diluted 1 : 10 with buffer, sonicated, and centrifuged for 20 min at 10,000 *g*. The supernatant fraction was adjusted to pH 5 with 1 M acetic acid while stirring rapidly. The resulting suspension was centrifuged and the pellet was rinsed with buffer, resuspended in 1.2 ml buffer, and stored at −18°.

The activity of PP-ribose-P synthetase was assayed using a one-step procedure in which phosphoribosyl pyrophosphate synthetase catalyzed the conversion of ribose 5-phosphate and ATP to PP-ribose-P and AMP, and adenine phosphoribosyltransferase catalyzed the conversion of PP-ribose-P and [^{14}C]adenine to [^{14}C]AMP. The amount of radioactivity in [^{14}C]AMP was used as a measure of PP-ribose-P synthetase activity. The incubation mixture contained the following components: ribose 5-phosphate (0.5 mM), ATP (0.6 mM), reduced glutathione (2 mM), sodium phosphate buffer (50 mM, pH 7.4), magnesium chloride (6 mM), [^{14}C]adenine (0.2 mM), Tris–hydrochloride buffer (100 mM, pH 7.4), PP-ribose-P synthetase, and adenine phosphoribosyltransferase, in a final volume of 105 μl. The mixture was incubated at 37° for 30 min and the reaction was stopped by addition of 25 μl of 4 M formic acid. The mixture was chromatographed and the total radioactivity in adenine nucleotides was measured. The concentration of ATP used gave the maximum rate of PP-ribose-P synthesis without inhibiting adenine phosphoribosyltransferase.

6-Phosphofrutokinase activity was measured using the method described by Racker [15], with certain modifications. The activity was determined at 25° in an assay system containing the following components: reduced glutathione (1 mM), triethanolamine–hydrochloride buffer (200 mM, pH 7.6), NADH (*ca.* 0.1 mM), fructose 6-phosphate (1.8 mM), ATP (1.1 mM), magnesium sulfate (1.4 mM), 6-phosphofructokinase, glycerol 3-phosphate dehydrogenase (2.6 units) triosephosphate isomerase (6.5 units), and fructose 1,6-bisphosphate aldolase (0.2 units). The final volume was brought to 1 ml with 154 mM NaCl. All the enzyme preparations were diluted with distilled water. The 6-phosphofructokinase preparation was diluted just prior to each experiment, since it loses activity at a substantial rate when stored in distilled water. The blank cuvette contained all the components except 6-phosphofructokinase. The decrease in absorbance at 340 nM was measured using a Beckman recording spectrophotometer.

5′-Deoxyadenosine had no effect on any of the assays described above.

RESULTS

The object of this study was to determine what biochemical effects might be exerted by an adenosine analog, 5′-deoxyadenosine, that is neither phosphorylated nor deaminated. The first step was to verify that 5′-deoxyadenosine indeed was not appreciably metabolized in the experimental system chosen—Ehrlich ascites tumor cells incubated *in vitro*. Although the 5′-position is not available for phosphorylation, the possibility was considered that 2′- or 3′-phosphate derivatives might be formed in the tumor cells. To test this, extracts of cells that had been incubated for 30 min with 1 mM 5′-deoxyadenosine were subjected to high pressure liquid chromatography. No evidence of any phosphorylated derivatives of 5′-deoxyadenosine was obtained; the sensitivity of the system was such that 0.1% phosphorylation to the 2′- or 3′-monophosphate could have been detected.

Although 5′-deoxyadenosine has been found not to be a substrate of purified adenosine deaminase [5], the possibility still was considered that it might be deaminated under the conditions of these experiments. To test this, the magnitude of one of the biochemical effects of 5′-deoxyadenosine (inhibition of accumulation of PP-ribose-P; see below) was compared in control cells and in cells that had been treated with an inhibitor of adenosine deaminase, deoxycoformycin; the conditions used were known to inhibit the deamination of adenosine in Ehrlich ascites tumor cells *in vitro* by more than 99 per cent [16]. Treatment with deoxycoformycin did not alter the magnitude of this effect of 5′-deoxyadenosine. Although this does not prove that there is absolutely no deamination of 5′-deoxyadenosine under the conditions of these studies, any deamination that might occur does not appear to contribute to its biochemical effects.

The next step in this study was to determine if 5′-deoxyadenosine inhibited any of the various pathways of purine ribonucleotide synthesis. The first process that was studied was nucleotide synthesis from [^{14}C]adenosine. 5′-Deoxyadenosine (1.0 mM) inhibited the incorporation of radioactive adenosine into GTP, ATP and total purine ribonucleotides by 74, 91 and 89 per cent respectively. This inhibition could have resulted from inhibition

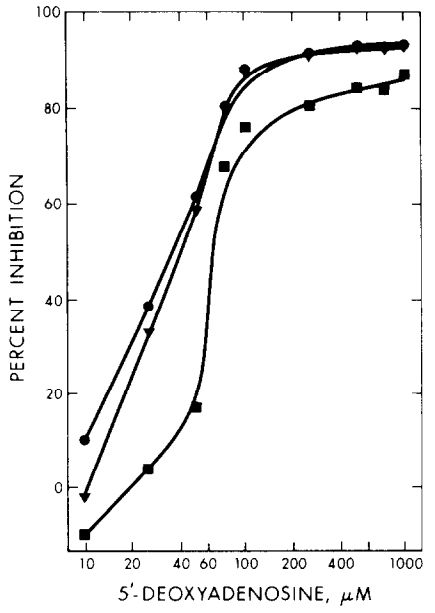

Fig. 1. Effect of 5′-deoxyadenosine on nucleotide formation from hypoxanthine. Ehrlich ascites tumor cells (2.0%, v/v) were incubated in modified Fischer's medium for 20 min at 37° with and without 5′-deoxyadenosine. [^{14}C]hypoxanthine was then added to a final concentration of 100 μM and the incubation was continued for an additional 30 min. Radioactivity in ATP (■), GTP (●) and total purine ribonucleotide (▼) was determined. Each point is the mean of results from duplicate determinations and is representative of results obtained in two experiments. Average deviation of individual analyses from the mean was less than 7 per cent.

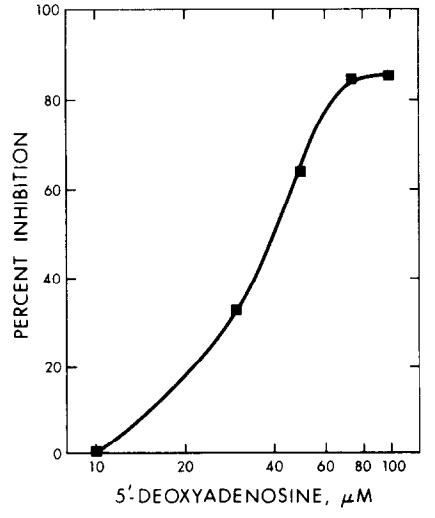

Fig. 2. Effect of 5′-deoxyadenosine on nucleotide formation from guanine. Tumor cells were incubated as described in Fig. 1 for 20 min, with and without 5′-deoxyadenosine, after which [^{14}C]guanine was added to a final concentration of 100 μM and incubation was continued for 30 min. Radioactivity in total purine nucleotides (■) was determined. Each point is the mean of results from duplicate determinations and is representative of results obtained in two experiments. Average deviation of individual analyses from the mean was less than 10 per cent.

of the entry of adenosine into the cells, inhibition of the phosphorylation of intracellular adenosine via adenosine kinase (EC 2.7.1.20), or by other mechanisms; attempts to distinguish among the various possibilities were not pursued.

Figures 1, 2 and 3 show that 5′-deoxyadenosine inhibited the synthesis of ribonucleotides from [^{14}C]hypoxanthine, [^{14}C]guanine and [^{14}C]adenine, respectively, and show the dose–response relationship for each process. The concentrations of 5′-deoxyadenosine that produced 50 per cent inhibition of these three processes were *ca.* 40, 40 and 300 μM respectively. In addition, Fig. 1 shows that GTP synthesis from [^{14}C]hypoxanthine was inhibited somewhat more extensively than was ATP synthesis.

Inhibition of purine biosynthesis *de novo* by 5′-deoxyadenosine is demonstrated in Fig. 4. This process was measured by determining the rate of incorporation of [^{14}C]-glycine into phosphoribosyl formylglycineamide in the presence of azaserine, an inhibitor of the conversion of this intermediate to phosphoribosyl formylglycineamidine. About 50 per cent inhibition was produced by 50 μM 5′-deoxyadenosine.

Slightly less inhibition of purine ribonucleotide synthesis by 5′-deoxyadenosine was observed when the period of incubation of cells and drug prior to addition of radioactive precursor was less than the usual 20 min. For example, 1 mM 5′-deoxyadenosine inhibited the incorporation of [^{14}C]hypoxanthine into ATP by 89 per cent when the first incubation was 20 min, whereas 79 per cent inhibition was

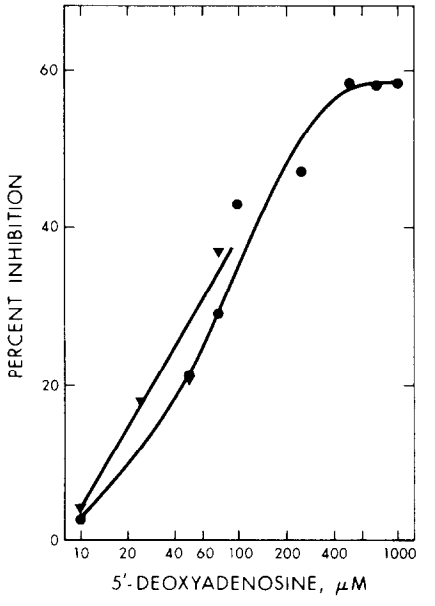

Fig. 3. Effect of 5′-deoxyadenosine on nucleotide formation from adenine. Tumor cells were incubated as described in Fig. 1. 1 for 20 min, with and without 5′-deoxyadenosine, after which [^{14}C]adenine was added to a final concentration of 100 μM and incubation was continued for 30 min. Radioactivity in ATP (●) and in total purine nucleotides (▼) was determined. Each point is the mean of results from duplicate determinations and is representative of results obtained in three experiments. Average deviation of individual analyses from the mean was less than 6 per cent.

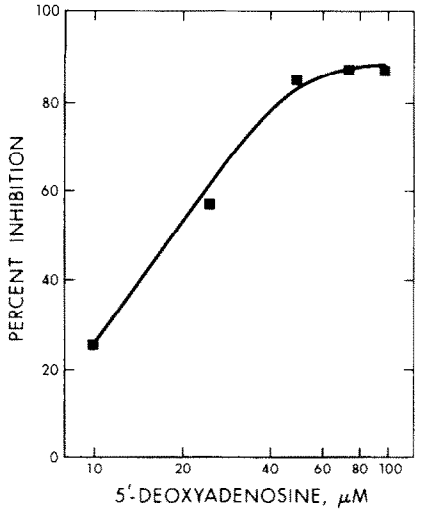

Fig. 4. Effect of 5′-deoxyadenosine on purine biosynthesis *de novo*. Tumor cells (2%, v/v) were incubated in Krebs–Ringer high phosphate medium containing 5.5 mM glucose and 10 μM azaserine for 10 min at 27° with and without 5′-deoxyadenosine. Glutamine and [^{14}C]glycine were then added to final concentrations of 1 mM each and incubation was continued for 50 min. Radioactivity in phosphoribosyl formylglycineamide (■) was then determined. Each point is the mean of results from duplicate determinations and is representative of results obtained in two experiments. Average deviation of individual analyses from the mean was less than 10 per cent.

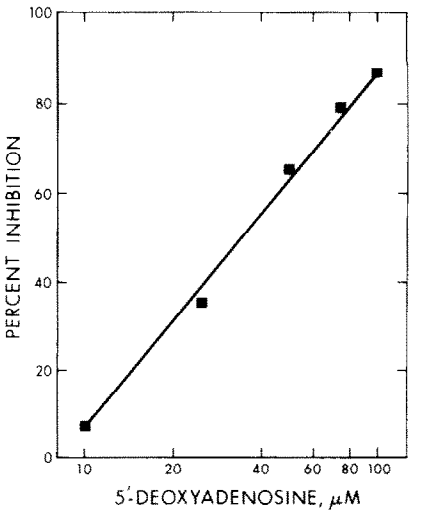

Fig. 5. Effect of 5′-deoxyadenosine on PP-ribose-P synthesis from glucose. Tumor cells (2%, v/v) were incubated in Krebs–Ringer high phosphate medium containing 5.5 mM glucose for 30 min at 37° with and without 5′-deoxyadenosine. Each point is the mean of results from duplicate determinations and is representative of results obtained in two experiments. Average deviation of individual analyses from the mean was less than 10 per cent.

obtained when the length of the first incubation was only 5 min.

One way in which 5′-deoxyadenosine might inhibit purine biosynthesis *de novo* as well as purine ribonucleotide synthesis from purine bases, would be to interfere with the synthesis or utilization of PP-ribose-P, a substrate that is required for all of these processes. As seen in Fig. 5, when Ehrlich ascites tumor cells were incubated under conditions in which PP-ribose-P utilization was virtually nil, the accumulation of PP-ribose-P was markedly inhibited by 5′-deoxyadenosine; 87 per cent inhibition was produced by 100 μM drug.

The most direct way by which 5′-deoxyadenosine could inhibit PP-ribose-P accumulation in tumor cells is by inhibition of PP-ribose-P synthetase. Therefore, this enzyme was partially purified from Ehrlich ascites tumor cells and assayed in the presence and absence of 5′-deoxyadenosine.

At the same time, another adenosine analog that is not phosphorylated but which also inhibits PP-ribose-P accumulation, 9-(3-aminopropyl)adenine [17], was also studied.

Table 1 shows that neither 0.6 nor 6.0 mM 5′-deoxyadenosine, in the presence of 0.5 mM ribose-5-P and 0.6 mM ATP, produced any detectable effect on PP-ribose-P synthetase activity. However, 10 mM 9-(3-aminopropyl)adenine inhibited the activity of this enzyme by 72 per cent. These results suggest that, although the previously observed effects of the latter compound are likely due to inhibition of PP-ribose-P synthetase, the effects of 5′-deoxyadenosine would seem to be due to some other mechanism.

Inasmuch as cyclic nucleotides have been implicated in the regulation of PP-ribose-P synthesis in at least one experimental system [18], the possibility that 5′-deoxyadenosine might mediate its effect on PP-ribose-P accumulation by increasing cyclic AMP concentrations was evaluated. First, as shown in Table 1, it was found that 100 μM cyclic AMP had little effect on the PP-ribose-P synthetase of Ehrlich ascites tumor cells. Thus, any elevation of cyclic AMP concentrations produced by 5′-deoxyadenosine would seem to have to operate at some other locus. However, when cyclic AMP concentrations in Ehrlich ascites tumor cells incubated with and without 1 mM 5′-deoxyadenosine were actually determined, these values were the same in both control and treated cells.

It has been demonstrated previously [7] that conditions which affect the rates of glycolysis and of

Table 1. Effect of 5′-deoxyadenosine and 9-(3-aminopropyl)adenine on PP-ribose-P synthetase*

Drug	Concn (mM)	Activity (per cent of control)
5′-Deoxyadenosine	0.6	104
5′-Deoxyadenosine	6.0	118
9-(3-Aminopropyl)adenine	10.0	27.6
Cyclic AMP	0.1	84.0

* PP-ribose-P synthetase was assayed using 0.5 mM ribose 5-phosphate, 0.6 mM ATP, 50 mM phosphate buffer, pH 7.4, and 6 mM $MgCl_2$, with and without 5′-deoxyadenosine, 9-(3-aminopropyl)adenine, or cyclic AMP, for 30 min at 37°. Control activity was 0.028 nmole PP-ribose-P formed/min. Results are averages of duplicate determinations and are representative of results of two experiments.

Table 2. Effect of 5′-deoxyadenosine on the formation of $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose*

Precursor	Time (min)	$^{14}CO_2$ (dis./min) Control	$^{14}CO_2$ (dis./min) 5′-Deoxyadenosine	Per cent of control
[1-^{14}C]glucose	5	7,090	8,022	113
	10	11,960	15,190	127
	15	20,040	24,190	121
[6-^{14}C]glucose	15	3,387	2,936	87
	30	4,863	4,590	94
	45	14,860	7,761	52

* Ehrlich ascites tumor cells (3.2%, v/v) were incubated in Krebs–Ringer phosphate medium containing 2 mM [^{14}C]glucose, with and without 0.1 mM 5′-deoxyadenosine. Results are averages of duplicate determinations and are representative of results of two experiments.

the pentose phosphate pathways can also affect the accumulation of PP-ribose-P in Ehrlich ascites tumor cells *in vitro*, presumably by decreasing the availability of ribose 5-phosphate for this process. Therefore, the effects of 5′-deoxyadenosine on several aspects of carbohydrate metabolism were studied.

The first point investigated was the effect of 5′-deoxyadenosine on the oxidative pentose phosphate pathway, as measured by $^{14}CO_2$ formation from [1-^{14}C]glucose. In addition, the overall process of glucose oxidation was studied by measuring $^{14}CO_2$ formation from [6-^{14}C]glucose. Table 2 shows that 5′-deoxyadenosine actually stimulated the oxidative pentose phosphate pathway. In contrast, whereas $^{14}CO_2$ formation from [6-^{14}C]glucose was not much affected during the first 30-min incubation, this was progressively inhibited thereafter. These observations indicated that 5′-deoxyadenosine did indeed have appreciable effects on various aspects of carbohydrate metabolism that might lead to inhibition of PP-ribose-P accumulation.

Further information on the effects of 5′-deoxyadenosine was obtained by measuring lactate accumulation during incubation of Ehrlich ascites tumor cells with glucose and with uridine. It has previously been observed that *ca.* 80 per cent of pyruvate produced glycolytically in these cells accumulated as lactate [7]. Table 3 shows that lactate formation from glucose was inhibited 38 per cent in cells incubated with 100 μM 5′-deoxyadenosine. The formation of lactate from uridine was also inhibited, though to a somewhat lesser degree (25 per cent); uridine is phosphorolyzed to ribose 1-phosphate which in turn is converted to ribose-5-P.

Table 3. Effects of 5′-deoxyadenosine on concentrations of glycolytic intermediates and lactate*

Intermediate	Concentration (per cent of control)
Glucose 6-phosphate	117
Dihydroxyacetone phosphate	95.4
Fructose 1,6-bisphosphate	71.4
Lactate	62.3

* Ehrlich ascites tumor cells (3.2%, v/v) were incubated in Krebs–Ringer phosphate medium containing 5.5 mM glucose for 30 min at 37° with and without 0.1 mM 5′-deoxyadenosine. Control values (in μmoles/ml packed cells) were: glucose 6-phosphate, 0.12; dihydroxyacetone phosphate, 0.160; fructose 1,6-bisphosphate, 208; and lactate, 50.2. Results are averages for duplicate determinations and are representative of results of two experiments.

The possibility that 5′-deoxyadenosine might generally affect the oxidation-reduction state of the cell was cursorily assessed by measuring its effect on lactate formation from pyruvate in cells incubated without glucose. 5′-Deoxyadenosine had essentially no effect on this process.

In further attempts to define the effects of 5′-deoxyadenosine on carbohydrate metabolism, concentrations of selected glycolytic intermediates were measured in control and treated cells. Incubation with 100 μM 5′-deoxyadenosine produced a 17 per cent increase in the concentration of glucose 6-phosphate, a 29 per cent decrease in that of fructose 1,6-bisphosphate, and essentially no change in the concentration of dihydroxyacetone phosphate. Unfortunately, concentrations of fructose 6-phosphate were too low to be accurately measured.

These results suggested the possibility that 5′-deoxyadenosine might affect the activity of phosphofructokinase. Table 4 shows first the well known stimulation of this enzyme by orthophosphate, and its inhibition by ATP. Both adenosine and 5′-deoxyadenosine also stimulated phosphofructokinase activity, and stimulation by 1.0 mM 5′-deoxyadenosine varied from 24 to 69 per cent when different enzyme preparations were used.

DISCUSSION

These studies have shown that a purine nucleoside analog that is not phosphorylated can have marked effects on cellular metabolism. Incubation of cells with 5′-deoxyadenosine was first found to inhibit the synthesis of purine ribonucletides *de novo* and via the adenine and hypoxanthine-guanine phosphoribosyltransferase reactions. Previous studies with other purine analogs have shown that inhibition of these distinct pathways of purine ribonucleotide synthesis can be mediated through inhibition of the synthesis of PP-ribose-P or by diverting PP-ribose-P into other pathways (review: Ref. 19). That this was highly likely in the case of 5′-deoxyadenosine was suggested both by failure of this compound to inhibit adenine phosphoribosyltransferase in cell extracts,

Table 4. Effect of 5′-deoxyadenosine, adenosine, ATP and phosphate on phosphofructokinase activity*

Experiment	Additions	Phosphofructokinase activity (per cent of control)
1	Phosphate (1 mM)	106
	Phosphate (10 mM)	144
2	ATP (6.6 mM)	35
	ATP (12.1 mM)	17
	ATP (6.6 mM) + 5′-deoxyadenosine (1.0 mM)	61
3	5′-Deoxyadenosine (1.0 mM)	169
4	5′-Deoxyadenosine (1.0 mM)	124
5	5′-Deoxyadenosine (1.0 mM)	124
	Adenosine (1.0 mM)	116

* Controls contained 1.8 mM fructose 6-phosphate, 1.1 mM ATP, and 1.4 mM $MgSO_4$. Separate controls were used for each experiment. The average control value from six experiments was 0.0019 μmole substrate consumed/min.

and also by the demonstration that PP-ribose-P synthesis was indeed inhibited in cells incubated with 5′-deoxyadenosine.

The observation that nucleotide synthesis from adenine was less sensitive to 5′-deoxyadenosine action than nucleotide synthesis *de novo* or from hypoxanthine and guanine has also been made with other purine analogs that inhibit the synthesis of PP-ribose-P [17, 20]. These differences usually are explained in terms of different Michaelis constants for the adenine phosphoribosyltransferase and hypoxanthine-guanine phosphoribosyltransferase reactions; however, the reported apparent Michaelis constants for the Ehrlich ascites tumor cell enzymes are of the same order of magnitude [21, 22]. This question, therefore, requires further investigation.

Some purine analogs that inhibit PP-ribose-P synthesis do so by directly inhibiting PP-ribose-P synthetase; this is true, for example, of xylosyl adenine and cordycepin, although these compounds must first be converted to their triphosphate derivatives. The free nucleosides decoyinine and psicofuranine also appear to inhibit PP-ribose-P synthetase in extracts of *Streptococcus faecalis* (review: Ref. 19) but this action has not been demonstrated using mammalian cells. In these studies, 5′-deoxyadenosine did not inhibit PP-ribose-P synthetase activity in cell extracts, although this enzyme was inhibited by another purine analog that could not be phosphorylated, 9-(3-aminopropyl)adenine.

Cyclic AMP has been reported to inhibit PP-ribose-P synthetase in some systems [18]. Although Wolff and Cook [23] found that 5′-deoxyadenosine did not stimulate adenylate cyclase from Y1 adrenal tumor cells, Blume and Foster [24] reported that it inhibited adenylate cyclase activity in mouse neuroblastoma cells, and Daly *et al.* [25, 26] have observed that 5′-deoxyadenosine stimulates cyclic AMP synthesis in slices of guinea pig cerebral cortex. In the present system, 5′-deoxyadenosine did not alter intracellular cyclic AMP concentrations and cyclic AMP did not affect PP-ribose-P synthetase activity.

These studies led to the hypothesis that 5′-deoxyadenosine inhibited the synthesis of PP-ribose-P in Ehrlich ascites tumor cells by inhibiting the conversion of glucose to ribose 5-phosphate, or by decreasing the availability of ribose-5-P for the PP-ribose-P synthetase reaction. Previous studies have shown that PP-ribose-P synthesis is affected, usually adversely, by a variety of conditions that affect carbohydrate metabolism in general [7]. Unfortunately, the exact relationship between PP-ribose-P synthesis and rates of glycolysis and of the oxidative and non-oxidative pentose phosphate pathways has not been defined in detail. The situation is complicated by the fact that even maximum rates of PP-ribose-P synthesis in these cells represent only *ca.* 2–3 per cent of total glucose utilization [7]. Hence at least potentially a small effect on major pathways might lead to a large effect on PP-ribose-P synthesis.

The present work has shown that 5′-deoxyadenosine inhibits lactate formation both from glucose and from uridine, and also stimulates the oxidative pentose phosphate pathway. Other investigators have shown that adenosine itself stimulates glycogen accumulation in some cultured cells [27], and inhibits gluconeogenesis in slices of rat liver [28]. In bacteria, another adenosine analog, tubercidin, is a potent inhibitor of glycolysis [29]. These observations suggest that adenosine and its analogs can have direct effects on major pathways of carbohydrate metabolism, but neither their specific sites of action nor the relationship of these to PP-ribose-P synthesis have yet been defined in any detail.

Finally, two other effects of 5′-deoxyadenosine may be discussed briefly. The fact that the conversion of radioactive hypoxanthine to guanine nucleotides was inhibited more than its conversion to adenine nucleotides is compatible with a weak inhibition of guanylate synthetase, as has been observed by Spector *et al.* [30, 31]. Inhibition of nucleotide formation from adenosine seems likely to be due to competition of adenosine and 5′-deoxyadenosine for uptake into cells, although other sites of action have not been ruled out.

Previous studies have shown that adenosine inhibits pyrimidine biosynthesis in some biological systems (e.g. Ref. 2). Although this point was not investigated in the present study, the observed inhibition of PP-ribose-P synthesis by 5′-deoxyadenosine would be expected to lead to inhibition of pyrimidine biosynthesis.

Although it has been suggested that adenosine

must be phosphorylated in order to be toxic [2, 3], at least in some biological systems, recent studies by Hershfield *et al.* [4] have shown that unmetabolized adenosine itself is toxic in certain human lymphoblast lines. This observation lends support to the relevance of the present studies of 5′-deoxyadenosine to the problem of adenosine toxicity.

Acknowledgement—We are grateful to Ms. Camilla M. Smith, who performed the cyclic AMP assays.

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