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Effects of dipyridamole on nucleotide synthesis from adenine in murine tumor cells*

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Dipyridamole (2,6-bisethanolamino-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine, Persantin) is a well-known inhibitor of nucleoside uptake into a variety of animal cells [1–13]. Recently, Zylka and Plagemann [14] reported that dipyridamole also inhibits the uptake of adenine, guanine and hypoxanthine into cultured Novikoff hepatoma cells, and this group has used these observations to support the hypothesis that purine base uptake in general is a mediated process [12]. In contrast, other workers previously had reported that dipyridamole had no effect or only slight effects on purine base uptake into primary cultures of chick fibroblasts [2], perfused guinea pig heart [4], human platelets [6] and murine leukemia L1210 cells [9].

In view of the importance of this point for understanding mechanisms of purine base uptake, we have investigated the effects of dipyridamole on nucleotide synthesis from adenine (i.e. adenine "uptake") in seven transplantable murine ascites tumor lines, when these cells were incubated *in vitro*.

Sources of most materials, methods of tumor cell preparation and incubation, and procedures for the separation and measurement of radioactivity in purine bases, ribonucleosides and ribonucleotides have been reported previously [15,16]. Ehrlich ascites tumor cells (Ehrlich) were carried in Swiss mice, Sarcoma 180 ascites tumor cells (S180) and Adenocarcinoma 755 ascites tumor cells (CA755) in BDF1 mice, 6C3HED lymphosarcoma ascites tumor cells (6C3HED) and Hepatoma 134 ascites tumor cells (H134) in C3H mice, leukemia L1210 ascites tumor cells (L1210) in DBA mice, and Mecca lymphosarcoma ascites tumor cells (Mecca) in AKDF1 mice. Dipyridamole was a gift of Drug Research and Development, National Cancer Institute, Bethesda, MD.

Inasmuch as intracellular pools of free purine bases and nucleosides usually have been found to be infinitesimal, studies of the effects of dipyridamole on purine base and nucleoside "uptake" usually have involved measurement of the incorporation of radioactive precursor into acid-soluble nucleotides, into nucleic acids, or both. Whether entry of adenine into the cell ("transport") is rate-limiting for the overall process of intracellular nucleotide formation ("uptake") is not established. Zylka and Plagemann [14] noted that more than 95 per cent of intracellular metabolites of adenine, hypoxanthine and guanine were nucleotides, and that vitually all of the nucleotide radioactivity was in ATP, GTP or both. Therefore, we have measured the incorporation of [^{14}C]adenine into acid-soluble purine ribonucleotides in the presence and absence of dipyridamole. In some experiments, radioactivity in all the purine ribonucleotides (ATP, ADP, GTP, GDP, adenylate, inosinate, xanthylate and guanylate) was measured, but since more than 96 per cent of the nucleotide radioactivity was in adenine nucleotides, in other experiments only radioactivity in ATP, ADP and adenylate was measured. Under the conditions used, less than 10 per cent of total cellular radioactivity was acid insoluble; therefore, little error is made by neglecting these data. At all concentrations of [^{14}C]adenine used, incorporation of radioactivity into acid-soluble nucleotides was linear during the 20-min period used.

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As Zylka and Plagemann [14] had emphasized the competitive nature of the inhibition of purine base "uptake" by dipyridamole, our studies were carried out using a fixed, relatively high concentration of dipyridamole (100 μM) and either a range of concentrations of [^{14}C]adenine (1–100 μM) or a single low concentration (10 μM) of [^{14}C]adenine. Because of the low precursor concentrations that sometimes were used, and to evaluate the possibility that effects of dipyridamole might not be linear with time, nucleotide synthesis was measured at three or four time points. Cells were incubated with dipyridamole for 20 min prior to addition of the radioactive adenine.

Table 1 (upper half) shows the effect of 100 μM dipyridamole on nucleotide synthesis from [^{14}C]adenine in Ehrlich ascites tumor cells both as a function of time and of adenine concentration. Inhibition by dipyridamole varied between 6 and 25 per cent. Inhibition did not seem to vary in a consistent way with time of incubation, and hence a mean of the separate values has been calculated. Although there was a general tendency toward less inhibition at the higher concentrations of adenine, it seems unlikely that such differences are of statistical significance. Thus, in contrast to the results of Zylka and Plagemann [14] with cultured hepatoma cells, the effects of dipyridamole in Ehrlich ascites tumor cells were slight, not progressive with time, and not competitive.

Less detailed studies were carried out using six other lines of transplanted ascites tumors (Table 1 lower half). In these cases a drug:adenine ratio of 10 was maintained throughout (Zylka and Plagemann [14] usually used 20 μM dipyridamole and 2.5 μM radioactive purine). Again inhibition was not progressive with time and was not great in magnitude. However, individual tumors exhibited different responses to dipyridamole with Mecca being the most sensitive (37 per cent inhibition) and L1210 the least sensitive (8 per cent inhibition). In other experiments, S180 and L1210 cells were also studied using 25, 50 and 100 μM [^{14}C]adenine, 100 μM dipyridamole, and a single incubation time (20 min). The results (not shown) were essentially the same as those shown in Table 1.

Especially because dipyridamole has been shown to be an inhibitor of mitochondrial electron transport [17, 18], the possibility was considered that it might have induced nucleotide breakdown, leading to misleading results when only radioactivity in nucleotides was being measured. To test this possibility, radioactivity in adenosine, inosine and hypoxanthine was measured in the experiments using Ehrlich ascites tumor cells, as these compounds are produced only by the breakdown of nucleotides synthesized from the [^{14}C]adenine. In control cells incubated for 20 min with 5, 10, 25, 50 or 100 μM [^{14}C]adenine, radioactivity in adenosine, inosine and hypoxanthine accounted for between 1.7 and 2.6 per cent of total nucleotide synthesis. Dipyridamole increased this percentage by between 1.5- and 2.8-fold, which indicates a small effect on energy metabolism. However, this slight acceleration in nucleotide breakdown could not account for the inhibition of nucleotide accumulation produced by dipyridamole.

We conclude that dipyridamole is not a potent inhibitor of adenine uptake in the seven ascites tumor lines used in this study, and that its slight effects were not competitive with respect to adenine; however, some differences were observed in the responses of individual cell lines to dipyri-

Table 1. Effects of dipyridamole on nucleotide synthesis from [^{14}C]adenine*

Tumor	[^{14}C]adenine (μM)	Nucleotide synthesis (per cent of control)				
		Time (min)				
		2.5	5	10	20	Mean ± S. D.
Ehrlich	1	75.3	81.5	84.8	73.7	78.8 ± 5.2
	5	68.3	83.7	81.7	85.3	79.7 ± 7.7
	10	73.8	70.4	88.6	83.9	79.1 ± 8.5
	25	80.4	81.3	77.9	82.9	80.6 ± 2.1
	50	84.9	82.9	81.5	88.3	84.4 ± 2.9
	100	93.7	84.6	91.4	90.9	90.1 ± 3.9
S180	10		89.5	72.7	78.3	80.2 ± 8.6
L1210	10		83.6	98.3	96.9	92.9 ± 8.1
6C3HED	10		73.4	82.9	85.3	80.5 ± 6.3
CA755	10		76.9	77.5	74.6	76.3 ± 1.5
H134	10		64.2	78.6	73.5	72.1 ± 7.3
Mecca	10		61.1	68.4	58.9	62.8 ± 4.9

* Ehrlich ascites tumor cells (2.0% suspension, v/v) were incubated in 2.0 ml of Fischer's medium containing 25 mM phosphate and an air atmosphere, with and without 100 μM dipyridamole. After 20 min, [^{14}C]adenine was added to the concentration indicated, and 100-μl samples were taken at the designated times. Other tumor cells were incubated in the same way, but the incubation volume was 100 μl, and separate samples were used for each time point. Neutralized perchloric acid extracts were chromatographed on polyethyleneimine-cellulose, and radioactivity was measured in individual purine ribonucleotides. The following are given as typical control values: Ehrlich ascites tumor cells incubated for 20 min with 1, 5, 10, 25, 50 and 100 μM [^{14}C]adenine synthesized 31.5, 220, 378, 601, 1185 and 1961 nmoles of radioactive nucleotides/g, respectively. Values reported are average of duplicate measurements and are representative of results obtained in two to three experiments. Within each experiment, the average deviation of individual analyses from the mean was less than 7 per cent.

damole. How it produces the slight inhibition observed is not known. These results in general correspond with previous studies in other systems [2, 4, 6, 9]. Whether the potent effects of dipyridamole observed in the studies of Zylka and Plagemann [14] were due to the cell line or the particular experimental condition used remains to be elucidated. However, studies using dipyridamole cannot be said, in general, to support the hypothesis that purine base uptake invariably is a mediated process.

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