The Effect of Adenosine N^1-Oxide on the Incorporation of Radioactive Precursors into the Nucleic Acids

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SUMMARY

Adenosine N^1-oxide is phosphorylated by Ehrlich ascites tumor cells to AMP N^1-oxide and ATP N^1-oxide. In Ehrlich cells incubated with adenosine N^1-oxide the incorporation of thymidine-^{3}H and ^{32}P into DNA is inhibited. The results indicate that AMP N^1-oxide or ATP N^1-oxide is responsible for the inhibition. The incorporation of ^{32}P and uridine-^{3}H into RNA is not inhibited by the analog. The inhibitory effect of adenosine N^1-oxide on the incorporation of thymidine-^{3}H into DNA can be abolished by the addition of 2′-deoxycytidine, cytidine, and to some extent uridine, but cytosine and 2′-deoxyguanosine have no effect on the inhibition. Adenosine N^1-oxide inhibits the incorporation of adenine-^{3}H and orotic acid-^{3}H into both DNA and RNA. In the case of adenine-^{3}H this is shown to be due partly to a decreased uptake of the tracer into the ATP pool.

INTRODUCTION

Adenosine N^1-oxide (1) inhibits the growth of neoplastic cells in tissue culture (2) and also has a slight inhibitory effect on growth of Ehrlich ascites cells *in vivo* (3). Recently it was shown that adenosine N^1-oxide can be phosphorylated to the triphosphate in a reaction catalyzed by myokinase and a purified adenosine kinase from rabbit liver (4). The formation of ATP N^1-oxide within the cells could be expected to affect the different processes in which ATP takes part. The present communication[1] describes the metabolism of adenosine N^1-oxide in Ehrlich ascites tumor cells and the effect of adenosine N^1-oxide on the incorporation of labeled precursors into DNA and RNA.

MATERIALS AND METHODS

Adenosine N^1-oxide was prepared by oxidation of adenosine with hydrogen peroxide in acetic acid as described by Stevens *et al.* (1). AMP N^1-oxide and ATP N^1-oxide were gifts from Professor H. Klenow. The two compounds were prepared by oxidation of AMP and ATP, respectively, with monoperphthalic acid (5).

The specific activity of RNA and DNA was determined by a modification of the procedure of Kit *et al.* (6). Cell suspension, 500 μl, was added to an equal volume of ice-cold 0.5 N perchloric acid. The precipitate was washed with 2 ml of the following solutions: three times with ice-cold 0.25 N perchloric acid, once with 90% alcohol (v/v) containing 2% sodium acetate (w/v) (7), and then once with 96% alcohol. The precipitate was suspended in alcohol–ether (3:1), heated at 65° for 5 min, cooled, centrifuged down, and washed once more with alcohol–ether. The pellet was digested with 500 μl of 0.5 N KOH at 37° for 16–18 hr and the solution was cooled in ice with one added volume of ice-cold 1 N perchloric acid. The supernatant was sucked off and the precipitate was washed twice with ice-cold 0.25 N trichloroacetic acid and finally heated in 1.0 ml of 0.25 N trichloroacetic

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acid at 90° for 15 min to hydrolyze DNA. Ribose was determined after Mejbaum (8) and deoxyribose after Burton (9). The radioactivity was measured in a liquid scintillation spectrometer, and the specific activity was expressed as counts per minute per micromole of ribose or deoxyribose.

The inhibition of incorporation was determined as follows. Aliquots were taken 45, 90, 135, 180 min after addition of the tracer, and the specific activity was determined as described above. The increase in specific activity with time was plotted, and the slopes of the two straight lines obtained for control cells and adenosine N^1-oxide-treated cells were used for calculation of the inhibition. Adenine-2,8-T, orotic acid-5-^{3}H, thymidine (methyl-^{3}H), and uridine-^{3}H(G) were purchased from The Radiochemical Centre, Amersham, England.

A hyperdiploid line of Ehrlich ascites tumor cells was maintained in NMRI mice by intraperitoneal injections of ascites fluid. The cells were harvested 7 days after transplantation.

Thin-layer chromatography. The plates with poly(ethyleneimine) (PEI) and MN 300 cellulose in 0.5-mm layer thickness were prepared and washed according to Randerath (10, 11):

Gradient 1: distilled water to 1.5 cm; 0.5 M ammonium formate, pH 3.2, to 1.8 cm; 2.0 ammonium formate, pH 3.2, to 3.0 cm; 4.0 M ammonium formate, pH 3.2, up to 13 cm.

Gradient 2: 1.0 M ammonium formate, pH 6.5, to 1.5 cm; 2.0 M ammonium formate, pH 6.5, to 4.5 cm; 4.0 M ammonium formate, pH 6.5, up to 12 cm.

Gradient 3: 2.0 M ammonium formate, pH 6.5, to 5 cm; 4.0 M ammonium formate, pH 6.5, up to 16 cm.

Ultraviolet absorbing spots were eluted with 1.5 ml 4.0 M LiCl or 0.7 M $MgCl_2$–2.0 M Tris hydrochloride, pH 7.4 (100:1 v/v) (12). Corrections were made for background absorption.

The amounts of adenine derivatives were determined from the absorbancy at 260 mμ assuming the molar extinction coefficient to be 15,000 $M^{-1}cm^{-1}$. The amounts of adenosine N^1-oxide derivatives were determined from the absorbancy at 233 mμ assuming the molar extinction coefficient to be 41,000 $M^{-1}cm^{-1}$ (ref. 13).

The metabolism of adenosine N^1-oxide in Ehrlich cells was analyzed as follows. After incubation of the cell suspensions for the time indicated, an aliquot was added to an equal volume of ice-cold 0.5 N perchloric acid. The samples were centrifuged and the supernatant neutralized with KOH. After precipitation of the $KClO_4$ at 0° and centrifugation, an appropriate amount of the $KClO_4$ supernatant was applied to PEI-cellulose plates. These were developed in the first dimension in a gradient of ammonium formate pH 3.2 (gradient 1) which separates nucleosides, mono-, di-, and triphosphates from each other. Before washing the plates in methanol, the adenosine N^1-oxide was eluted, as it would be washed out by methanol. The plates were then developed in the second dimension in a gradient of ammonium formate, pH 6.5 (gradient 2), which separates the adenosine N^1-oxide phosphate esters from the corresponding adenosine phosphates (Fig. 1). Identical chromatograms were made from the acid-soluble fraction of control cells.

In some cases ATP was measured after development of the plates in one dimension in gradient 3, since the slow migrating ATP is well separated from the rest of the nucleotides.

Paper chromatography. The purine and pyrimidine bases and nucleosides present in the acid-soluble fraction of Ehrlich cells were isolated by paper chromatography. After neutralization of this fraction with KOH at 0° and removal of $KClO_4$ by centrifugation, an appropriate amount of the supernatant was applied on Whatman No. 40 filter paper strips (width 2 cm) and the chromatograms were developed in *n*-butanol saturated with water (14) for 21 hr. The strips were cut into 2-cm pieces which were counted in a liquid scintillation spectrometer.

RESULTS

The metabolism of adenosine N^1-oxide in Ehrlich cells was studied by thin-layer

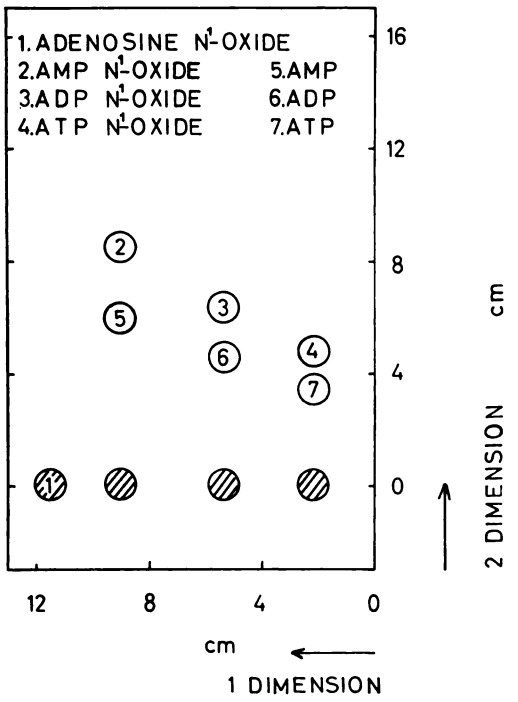

FIG. 1. *The chromatographic separation of adenosine N^1-oxide and its phosphate esters from each other and from the corresponding adenosine phosphates*

PEI-cellulose plates were developed in a gradient of ammonium formate pH 3.2 (gradient 1) in the first dimension and in a gradient of ammonium formate pH 6.5 (gradient 2) in the second dimension. For details see Materials and Methods.

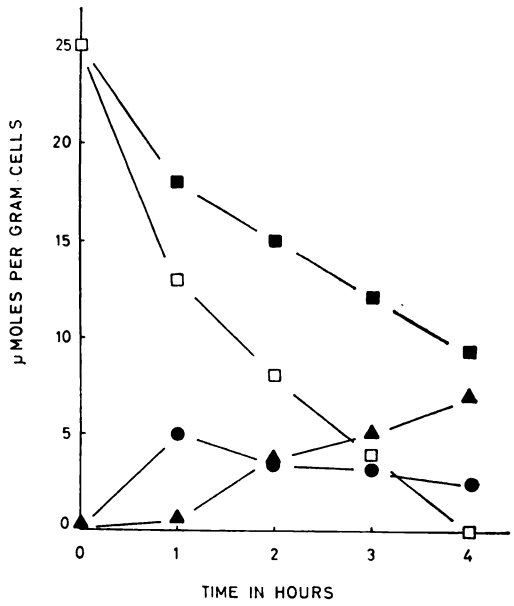

FIG. 2. *The metabolism of adenosine N^1-oxide in Ehrlich ascites cells in vitro*

The reaction mixture contained per milliliter: 80 mg of Ehrlich ascites cells (wet weight), 420 μl of cell-free ascites fluid, 500 μl of Robinson's medium (17) which was 5.6 mM with respect to glucose, 20 mμmoles of folate (18), and 2.0 μmoles of adenosine N^1-oxide. The acid-soluble fraction of the cell suspension was analyzed by thin-layer chromatography on PEI-cellulose plates as described in Materials and Methods. □—□, Adenosine N^1-oxide; ●—●, AMP N^1-oxide; ▲—▲, ATP N^1-oxide; ■—■, sum of adenosine N^1-oxide, AMP N^1-oxide, and ATP N^1-oxide.

chromatography. After application of an aliquot of the acid-soluble fraction on PEI-cellulose plates these were developed in two dimensions as seen on Fig. 1. The concentration of adenosine N^1-oxide dropped rapidly while two phosphorylated derivatives of adenosine N^1-oxide accumulated (Fig. 2). These two compounds were identified as AMP N^1-oxide and ATP N^1-oxide by comparison with synthetic AMP N^1-oxide and ATP N^1-oxide. The two phosphorylated compounds accumulating in Ehrlich cells were eluted from the PEI-cellulose plates, and the spectrum of the eluate was the characteristic adenosine N^1-oxide spectrum. Furthermore the compounds cochromatographed with synthetic AMP N^1-oxide and ATP N^1-oxide, respectively, when chromatographed on PEI-cellulose plates in the three gradients described in Materials and Methods. No ADP N^1-oxide accumulated in Ehrlich cells incubated with adenosine N^1-oxide. The sum of adenosine N^1-oxide, AMP N^1-oxide, and ATP N^1-oxide decreased with time. This is probably due to a reduction of adenosine N^1-oxide to adenosine, analogous to the reduction that has been demonstrated for 2′-deoxyadenosine N^1-oxide and 3′-deoxyadenosine N^1-oxide (15, 16).

The inhibitory effect of adenosine N^1-oxide on the incorporation of thymidine-^{3}H into DNA is shown on Fig. 3 (upper part). The simultaneous accumulation of AMP N^1-oxide and ATP N^1-oxide is shown on the lower part of Fig. 3. Even though dif-

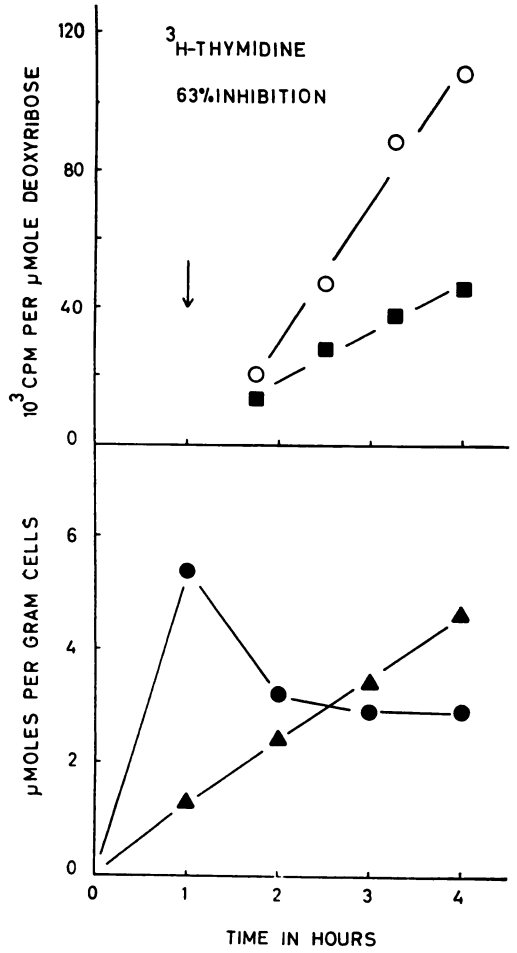

FIG. 3. *The inhibitory effect of adenosine N^1-oxide phosphate esters on the incorporation of thymidine-^{3}H into DNA*

Reaction mixtures were as described in Fig. 2, and 8.3 μC Thymidine-^{3}H (specific activity 20 μC/μmole) was added after 1 hr of preincubation with adenosine N^1-oxide as indicated by arrow on upper part of the figure. ○—○, Control; ■—■, adenosine N^1-oxide (2.0 μmoles/ml). *Lower part:* accumulation of AMP N^1-oxide (●—●) and (▲—▲) ATP N^1-oxide in the same cells.

ferent batches of Ehrlich ascites cells were treated identically, they accumulated AMP N^1 oxide and ATP N^1-oxide to a different extent (Table 1). Increasing accumulation fo these phosphate esetrs gave rise to an increasing inhibition of incorporation of radioactive precursors into DNA (Table 1). An experiment was therefore performed in order to find the conditions for maximal inhibition. Ehrlich cells were preincubated for increasing lengths of time with adenosine N^1-oxide. This would allow increasing concentration of ATP N^1-oxide and AMP N^1-oxide to be formed before addition of thymidine-^{3}H, and this should then give rise to increasing inhibition of incorporation of the tracer into DNA. Figure 4 demonstrates the positive correlation between the duration of preincubation with adenosine N^1-oxide and the inhibitory effect on the incorporation of thymidine-^{3}H into DNA.

The presence of adenosine N^1-oxide in Ehrlich ascites tumor cells inhibits the incorporation of ^{32}P, thymidine-^{3}H, adenine-^{3}H, and orotic acid-^{3}H into DNA from 37% to 81% (Table 2). It has been demonstrated (Table 1) that the inhibition of incorporation varied from cell batch to cell batch probably owing to a different extent of formation of adenosine N^1-oxide phosphates. In order to clarify whether the incorporation of ^{32}P and thymidine-^{3}H was inhibited to the same extent, or whether more than one process was inhibited by adenosine N^1-oxide, the inhibition of ^{32}P and thymidine-^{3}H incorporation into DNA was compared in a double-labeling experiment (Fig. 5). The incorporation of ^{32}P and thymidine-^{3}H was inhibited to the same extent. A different experiment was performed with one cell batch and conditions as described in Fig. 5 except that one flask contained thymidine-^{3}H and a different flask contained ^{32}P and unlabeled thymidine in the same concentration as the tritiated thymidine (0.24 μmole/ml). The presence of adenosine N^1-oxide also in this case inhibited the incorporation of the two tracers to the same extent (results not shown).

Adenosine N^1-oxide did not inhibit the incorporation of ^{32}P or uridine-^{3}H into RNA from Ehrlich cells. The incorporation of orotic acid-^{3}H and adenine-^{3}H into RNA was inhibited, but less than was the incorporation into DNA (Table 2). These findings could indicate that more than one process was inhibited by adenosine N^1-oxide. Since adenine-^{3}H (and orotic acid-^{3}H) react with PRPP as the first step in

TABLE 1

Accumulation of AMP N^1-oxide and ATP N^1-oxide and the corresponding inhibition of incorporation of radioactive tracers into DNA

Reaction mixtures were as described in Fig. 2. Thymidine-^{3}H, 8.3 μC (specific activity 20 μC/μmole) was added after 1 hr of preincubation with adenosine N^1-oxide (2.0 μmole/ml). ^{32}P, 18.0 μC, was added to the cells simultaneously with adenosine N^1-oxide For determination of inhibition see Materials and Methods.

		Micromoles per gram cells (wet wt.)								
		AMP N^1-oxide				ATP N^1-oxide				
Expt. No.	Tracer	1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr	Inhibition (%)
1	^{32}P	2.1	2.7	2.2	1.8	1.1	2.0	2.5	3.0	36
2	Thymidine-^{3}H	5.4	3.2	2.9	2.8	1.3	2.4	3.4	4.6	63
3	Thymidine-^{3}H	4.9	3.5	3.2	2.3	0.3	3.6	5.2	7.0	79

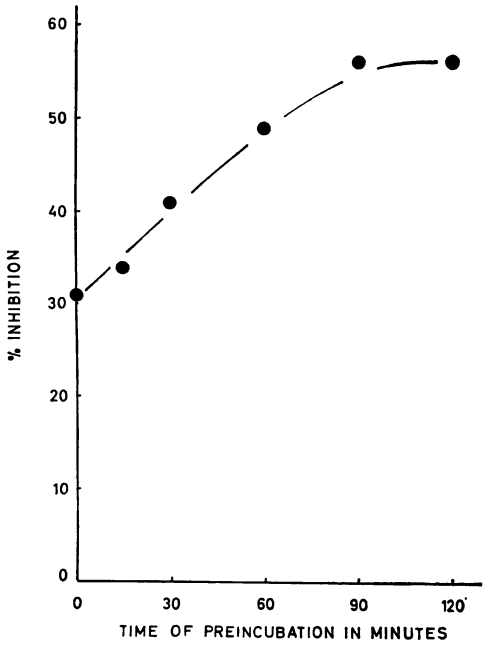

FIG. 4. *The inhibition of the incorporation of thymidine-^{3}H into DNA correlated to the length of preincubation of Ehrlich cells with adenosine N^1-oxide*

Reaction mixtures as described in Fig. 2. The cell suspensions were preincubated with adenosine N^1-oxide (2.0 μmoles/ml) for varying lengths of time as indicated, and 8.3 μC of thymidine-^{3}H (specific activity 20 μC/μmole) was then added. Aliquots were in each case taken 45, 90, 135, and 180 min after addition of thymidine-^{3}H, and the inhibition was determined as described in Materials and Methods.

the incorporation into nucleic acids, the incorporation of adenine-^{3}H into the ATP pool was investigated. A fraction of the acid-soluble pool of the cells was chromatographed on PEI-cellulose plates in gradient 3. In the presence of adenosine N^1-oxide the incorporation of adenine-^{3}H (tracer amounts) into the ATP pool is inhibited to about 50% of the control (Fig. 6). Spectrophotometric measurements showed that the concentration of ATP in the cells was constant, but since the specific activity in the ATP pool is about 50% lower in adenosine N^1-oxide-treated cells than in control cells, this will explain that the incorporation of adenine-^{3}H into RNA is inhibited. From this type of experiment, it cannot be decided whether it is the formation of PRPP or the reaction between adenine and PRPP that is inhibited. The fate of adenine-^{3}H was, therefore, further investigated. ATP was isolated after thin-layer chromatography of the acid-soluble fraction in gradient 3. Inosine, hypoxanthine, adenine, and xanthosine, were separated by paper chromatography, and the radioactivity in each of these spots was determined. The results are shown in Table 3. It is seen that adenine-^{3}H is metabolized much more rapidly in adenosine N^1-oxide-treated cells than in control cells. It is also seen from Table 3 that the incorporation of adenine-^{3}H into ATP is less in adenosine N^1-oxide-treated cells than in control cells. In the hypoxanthine,

TABLE 2

The inhibitory effect of adenosine N^1-oxide on the incorporation of different precursors into nucleic acids from Ehrlich ascites cells

The reaction mixtures were as described in Fig. 2. The reaction mixtures were preincubated with adenosine N^1-oxide (2.0 μmoles/ml) for 1 hr before addition of tracers in the following amounts: 15.0 μC ^{32}P, 8.3 μC thymidine-^3H (20.0 μC/μmole), 16.6 μC uridine-^3H (16.0 μC/μmole), 23.2 μC adenine-^3H (2.36 mC/μmole), 8.3 μC orotic acid-^3H (20.0 μC/μmole). For determination of inhibition see Materials and Methods.

Precursor	DNA inhibition	RNA inhibition	Number of experiments
^{32}P	50% (range 40–81)	0	5
Thymidine-^3H	63% (range 45–81)	—	9
Uridine-^3H	—	0	1
Adenine-^3H	37–59%	25–33%	2
Orotic acid-^3H	65%	25%	1

inosine, and xanthosine spot, the labeling is 5–10 times higher in adenosine N^1-oxide-treated cells than in control cells.

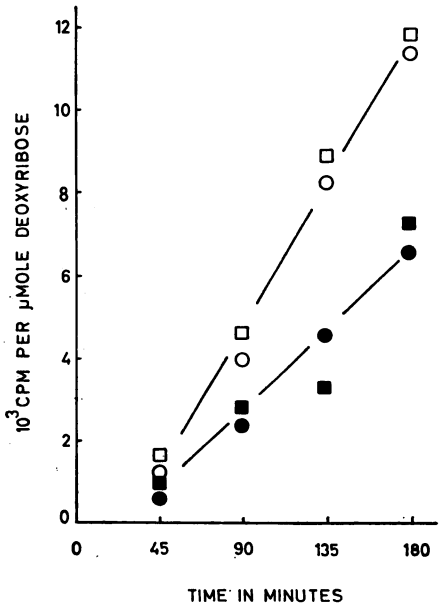

FIG. 5. *Double-labeling experiment showing the inhibitory effect of adenosine N^1-oxide on the incorporation of thymidine-^3H and ^{32}P into DNA*

The reaction mixtures were as described in Fig. 2. The cell suspensions were preincubated with adenosine N^1-oxide (2.0 μmoles/ml) for 1 hr before addition of 8.3 μC of thymidine-^3H (20 μC/μmole) and 3.7 μC of ^{32}P per milliliter. ○—○, Control (thymidine-^3H); ●—●, adenosine N^1-oxide (thymidine-^3H); □—□, control (^{32}P); ■—■, adenosine N^1-oxide (^{32}P).

It was found that the incorporation of ^{32}P into DNA was inhibited whereas the incorporation into RNA was not (Table 2). These findings could indicate that adenosine N^1-oxide or the phosphate esters inhibit the reduction of ribotides to 2′-deoxyribotides or a later step leading to DNA synthesis. By the addition of 2′-deoxynucleosides to adenosine N^1-oxide-

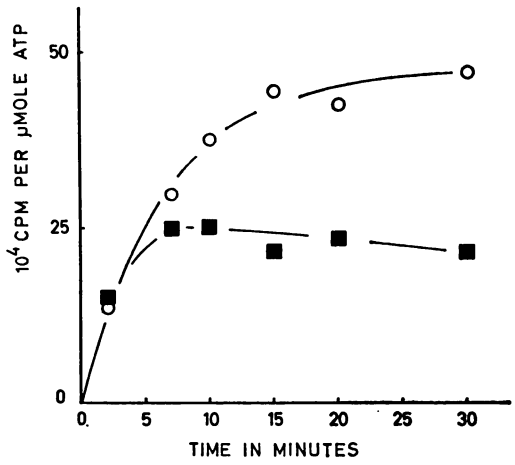

FIG. 6. *The inhibitory effect of adenosine N^1-oxide on the incorporation of adenine-^3H into the ATP pool*

The reaction mixtures were as described in Fig. 2. The cells were preincubated with adenosine N^1-oxide (2.0 μmoles/ml) for 1 hr before addition of 23.0 μC of adenine-^3H (2.36 mC/μmole) per milliliter of reaction mixture. ○—○, control; ■—■, adenosine N^1-oxide.

TABLE 3

The metabolism of adenine-^{3}H in Ehrlich cells treated with adenosine N^1-oxide

The reaction mixtures were as described in Fig. 2. The cells were preincubated with adenosine N^1-oxide (2.0 μmoles/ml) for 1 hr before addition of 12.0 μC adenine-^{3}H (2.36 mC/μmole) per milliliter of reaction mixture. Aliquots were taken 2, 5, 10, and 15 min after addition of adenine-^{3}H and the acid-soluble fraction was analyzed as described in Materials and Methods.

Compound	Sample	Radioactivity (× 10^{-4} cpm)			
		2 min	5 min	10 min	15 min
Adenine	Control	27.0	10.3	2.2	0.2
	Adenosine N^1-oxide	5.8	1.7	1.0	0.1
ATP	Control	18.8	19.9	24.5	29.1
	Adenosine N^1-oxide	8.4	9.3	9.8	13.1
Inosine	Control	4.1	3.8	3.8	3.9
	Adenosine N^1-oxide	19.0	21.5	19.5	17.5
Hypoxanthine	Control	2.3	2.0	0.2	0.2
	Adenosine N^1-oxide	14.5	15.6	14.6	13.2
Xanthosine	Control	—	—	0.1	0.2
	Adenosine N^1-oxide	0.2	2.6	2.4	1.7

TABLE 4

Effect of nucleosides and bases on the inhibition of incorporation of thymidine-^{3}H into DNA

The reaction mixtures were as described in Fig. 2. The cells were preincubated 1 hr with adenosine N^1-oxide (2.0 μmoles/ml) before addition of 7.7 μC thymidine-^{3}H (80 μC/μmole) and the compounds listed below in concentration of 1.0 μmole (a) and 0.4 μmole (b) per milliliter of reaction mixture. The experiments were performed in duplicate, and the results were normalized to the same control, the one without additions.

Additions	Relative specific activity of DNA
None	100
Adenosine N^1-oxide	49
Adenosine N^1-oxide + 2′-deoxyguanosine (a) and 2′-deoxycytidine (a)	100
Adenosine N^1-oxide + 2′-deoxycytidine (a)	100
Adenosine N^1-oxide + 2′-deoxyguanosine (a)	50
Adenosine N^1-oxide + cytidine (b)	100
Adenosine N^1-oxide + cytosine (b)	47
Adenosine N^1-oxide + uridine (b)	68

treated cells, thereby bypassing the possibly inhibited process, an attempt was made to localize the site of inhibition. Addition of a mixture of all four 2′-deoxynucleosides together with adenosine N^1-oxide did actually stimulate the incorporation of thymidine-^{3}H into DNA about 30–40% compared with a control with the four 2′-deoxynucleosides (2 experiments, not shown). Addition of 2′-deoxyguanosine plus 2′-deoxycytidine to adenosine N^1-oxide treated cells completely abolished the inhibition. 2′-deoxycytidine alone had the same effect, but 2′-deoxyguanosine could not abolish the inhibition (Table 4). Similar experiments were performed with cytidine, cytosine, and uridine to make clear whether the effect of 2′-deoxycytidine was specifically on the deoxyribonucleotide pathway. Addition of cytidine to adenosine N^1-oxide treated cells completely prevented inhibition of thymidine-^{3}H incorporation into DNA, whereas uridine had a small effect and cytosine could not abolish the inhibition.

DISCUSSION

When Ehrlich ascites tumor cells are incubated with adenosine N^1-oxide, this compound is partly phosphorylated and partly reduced. Between 5 and 10 μmoles of AMP N^1-oxide and ATP N^1-oxide ac-

cumulated per gram of cells, wet weight, whereas no ADP N^1-oxide could be detected. A large fraction of the adenosine N^1-oxide is reduced since the total amounts of N^1-oxide present as adenosine N^1-oxide plus the mono-, di-, and triphosphate decreased to less than half within 4 hr. It is not known whether the reduction of the N^1-oxide takes place at the nucleoside level or at the nucleotide level. It has previously been shown that 2′-deoxyadenosine N^1-oxide and 3′-deoxyadenosine N^1-oxide are reduced at the nucleoside level (15, 16).

The incorporation of thymidine-^{3}H and ^{32}P into DNA was inhibited by adenosine N^1-oxide whereas the incorporation of ^{32}P and uridine-^{3}H into RNA was not. This finding could indicate a specific effect on the reduction of ribotides to 2′-deoxyribotides or a later step leading to DNA synthesis. It has previously been demonstrated by Klenow (19) that 2′-deoxyATP inhibits DNA synthesis in Ehrlich cells because the compound inhibits the reduction of CDP and GDP to the corresponding deoxyribotides. The inhibition of DNA synthesis could be reversed by addition of 2′-deoxycytidine and 2′-deoxyguanosine, thereby bypassing the inhibited process. An attempt was also made to localize the inhibited process by addition of 2′-deoxynucleosides. It was found that 2′-deoxycytidine could completely abolish the inhibitory effect of the adenosine N^1-oxide, but that cytidine had the same effect (Table 4). Uridine had a slight effect and cytosine could not abolish the inhibition. From these results it is therefore not possible to conclude whether the reduction of ribotides to deoxyribotides is inhibited or not.

The decreased incorporation of adenine-^{3}H into the ATP pool of adenosine N^1-oxide-treated cells (Fig. 6) is presumably not due to an inhibition of the reaction with PRPP, as seen from the more rapid disappearance of adenine-^{3}H in adenosine N^1-oxide-treated cells than in control cells and the more rapid appearance of labeling in xanthosine, inosine, and hypoxanthine (Table 3). It seems most likely that ^{3}H-adenine is converted to AMP and that a large fraction of this compound is then rapidly (within 5 min) degraded to nucleosides and hypoxanthine. A fraction of the AMP is, however, phosphorylated to ATP and the ^{3}H-adenine incorporated into ATP disappears much more slowly (Fig. 6). The degradation of AMP to nucleosides and hypoxanthine is shown to occur also under conditions of glucose phosphorylation (20). Barclay and Phillipps (21) have shown that adenosine N^1-oxide inhibits the incorporation of adenine-^{14}C into nicotinic acid adenine dinucleotide and nicotinamide adenine dinucleotide whereas the incorporation of nicotinic acid-^{14}C and nicotinamide-^{14}C was not inhibited. This agrees well with the decreased incorporation of adenine-^{3}H into ATP described in the present communication.

The inhibition of the incorporation of ^{32}P and thymidine-^{3}H into DNA seems to be exerted by AMP N^1-oxide or ATP N^1-oxide rather than by adenosine N^1-oxide itself. The results presented in Table 1 and in Fig. 4 show that increasing accumulation of AMP N^1-oxide and ATP N^1-oxide gave rise to an increasing inhibition of incorporation. In agreement with these results, Bennett *et al.* (22) have shown that H.Ep#2 cells in culture are inhibited by adenosine N^1-oxide and that H.Ep#2 cells devoid of adenosine kinase activity were resistant to adenosine N^1-oxide.

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