Effect of 1-amino-oxy-3-aminopropane on polyamine metabolism and growth of L1210 cells

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1-Amino-oxy-3-aminopropane (AOAP) was reported to inhibit several mammalian polyamine-biosynthetic enzymes *in vitro*, including ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) [Khomutov, Hyvönen, Karvonen, Kauppinen, Paalanen, Paulin, Eloranta, Pajula, Andersson & Pösö (1985) *Biochem. Biophys. Res. Commun.* **130**, 596–602]. In order to clarify its mechanism of action in intact cells, the inhibitory properties of AOAP on the growth and polyamine metabolism of L1210 cells were compared with those seen in a variant subline (D-R cells) which overproduces ODC. As little as 20 μ M-AOAP completely blocked proliferation of L1210 cells, and this effect was reversed by the concomitant addition of exogenous putrescine or spermidine. Growth of D-R cells was not affected by AOAP at concentrations up to 0.5 mM. There was no difference in the uptake of AOAP between the L1210 and the D-R cells. Exposure of L1210 or D-R cells to AOAP greatly decreased ODC activity in undialysed cell extracts, but did not decrease AdoMetDC. Activities of both enzymes were increased severalfold by AOAP treatment when activity was measured in dialysed extracts. Treatment with AOAP depleted intracellular putrescine and spermidine contents of L1210 cells, while inducing a massive accumulation of decarboxylated AdoMet. The 8-fold higher putrescine pool present in untreated D-R cells was depleted in a dose-dependent manner by AOAP, but a significant decrease in spermidine and accumulation of decarboxylated AdoMet required 10 times higher drug concentrations, and the changes were much less dramatic than in L1210 cells. These results indicate that in L1210 cells AOAP behaves primarily as a reversible inhibitor of ODC.

INTRODUCTION

Khomutov and colleagues have reported that 1-amino-oxy-3-aminopropane (AOAP) is a potent inhibitor of polyamine biosynthesis that blocks the activities of three of the enzymes in the mammalian polyamine-biosynthetic pathway, namely ornithine decarboxylase (ODC), *S*-adenosylmethionine decarboxylase (AdoMetDC) and spermidine synthase (Khomutov *et al.*, 1985a). Since the polyamine-biosynthetic pathway is an attractive target for the design of anti-neoplastic agents (Porter & Sufrin, 1986; McCann *et al.*, 1987; Pegg, 1988), such inhibition raises the possibility that AOAP or related substances might be of value for this purpose and for investigation of the role of polyamines in neoplastic cell growth. At present, a number of potent and highly specific inhibitors of ODC are available, including α -difluoromethyl-ornithine (DFMO), and several compounds are under active investigation as potentially useful inhibitors of AdoMetDC or the aminopropyltransferases involved in polyamine synthesis (Pegg & McCann, 1982; Porter & Sufrin, 1986; Pegg, 1986, 1988; McCann *et al.*, 1987), but a general inhibitor of polyamine synthesis active against several enzymes in the pathway would be of considerable interest. However, the specificity of AOAP towards polyamine biosynthesis has not yet been firmly established, and there are few details on the potency with which this compound leads to the depletion of polyamines

and to the inhibition of tumour cell growth. Hyvönen *et al.* (1988) reported that 0.5 mM-AOAP prevented polyamine synthesis and blocked proliferation of cultured baby-hamster kidney cells and that these effects could be reversed by provision of 0.5 mM-putrescine or 0.1 mM-spermidine, but no dose-response studies were carried out. Both ODC and AdoMetDC activities were enhanced by exposure to the drug when these were assayed in dialysed extracts to remove inhibitors of low molecular mass.

We have studied the effects of AOAP on the polyamine metabolism and growth of L1210 cells and of an L1210 variant cell line (D-R cells) that is resistant to DFMO by virtue of a massive overproduction of ODC (Pegg *et al.*, 1988a).

MATERIALS AND METHODS

Materials

AOAP was synthesized as its dihydrochloride salt as described by Khomutov *et al.* (1985b). DFMO was generously given by Merrell Dow Research Institute, Cincinnati, OH, U.S.A. Synthetic decarboxylated AdoMet was kindly provided by Dr. K. Samejima, Josai University, Japan. *S*-Adenosyl[carboxy- 14 C]methionine (54 Ci/mol) and L-[1- 14 C]ornithine (51.6 Ci/mol) were purchased from Amersham Corp., Arlington Heights,

Abbreviations used: AOAP, 1-amino-oxy-3-aminopropane; AdoMet, *S*-adenosylmethionine; AdoMetDC, AdoMet decarboxylase; DFMO, α -difluoromethylornithine; ODC, ornithine decarboxylase.

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IL, U.S.A., and New England Nuclear, Boston, MA, U.S.A., respectively. All biochemical reagents were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Assay of ODC and AdoMetDC activities

ODC and AdoMetDC activities were determined by measuring the release of $^{14}\text{CO}_2$ from L-[1- ^{14}C]ornithine and [carboxy- ^{14}C]AdoMet, respectively, as described previously (Seely *et al.*, 1982; Pegg, 1984). A portion of the cell extracts prepared for the determination of enzyme activities was dialysed overnight against two 1-litre changes of the buffer used for homogenization, which was 50 mM-Tris/HCl (pH 7.5)/2.5 mM-dithiothreitol/0.1 mM-EDTA for ODC samples and 50 mM-sodium phosphate (pH 7.5)/2 mM-putrescine/1.25 mM-dithiothreitol/0.1 mM-EDTA for AdoMetDC samples. Extracts from the D-R cells were diluted 20-fold before assay of ODC activity. One unit of decarboxylase activity was defined as the amount needed to release 1 nmol of CO_2 /60 min, and results are expressed as units/mg of protein. Protein was determined by the method of Bradford (1976).

Cell culture

Control L1210 cells were routinely grown as a suspension in RPMI 1640 medium containing 10% (v/v) Nu-Serum (Collaborative Research, Lexington, MA, U.S.A.) as previously described (Porter *et al.*, 1987a). DFMO-resistant (D-R) cells derived from the L1210 cell line (Pegg *et al.*, 1988a) were grown in the same medium, except that 10 mM-DFMO was added routinely to stock cultures. Before experiments, both control and D-R cells were collected by centrifugation, washed once with culture medium and grown for 72 h in the absence of DFMO. Additions of test compounds to growth medium were always made at the time of cell seeding (i.e. at zero time of incubation). Aminoguanidine (1 mM) was added to media used for experiments in order to inhibit the action of serum oxidases. Cell number was determined with a haemocytometer.

Analysis of polyamines and decarboxylated AdoMet

Cells were harvested by centrifugation, washed with 10 ml of phosphate-buffered saline, and extracted with 3% (v/v) HClO_4 (Pegg, 1984). Samples of the filtered extracts were used for polyamine analysis by h.p.l.c. using an ion-pair reversed-phase separation system (Seiler & Knödgen, 1980). Contents of putrescine, spermidine and spermine were determined by post-column derivative formation with *o*-phthalaldehyde as described by Seiler & Knödgen (1985). Decarboxylated AdoMet was simultaneously determined in the same system by monitoring the A_{254} of the eluate, and comparing peak heights with those obtained with known amounts of the authentic compound.

Determination of intracellular content of AOAP

L1210 and D-R cell cultures were grown as described above and re-seeded in 50 ml of medium at 1×10^5 cells/ml in the absence or presence of 0.2 mM-AOAP. After 48 h the cells were collected by low-speed centrifugation and the pellets washed twice with 50 ml of ice-cold phosphate-buffered saline. The cell pellets were then resuspended in 0.9 ml of 50 mM-Tris/HCl (pH 7.5)/2.5 mM-dithiothreitol/100 μM -EDTA and lysed by freeze-thawing with

liquid N_2 . Cytosol extracts were obtained after a 15 min centrifugation at 17000 *g* at 4 °C. From measurements of [^{14}C]sucrose space, it was estimated that the contribution of extracellular AOAP could not exceed 0.1 nM in these final cell extracts. To measure the intracellular contents of AOAP, the cytosol extracts were heated at 100 °C for 5 min, cooled on ice and centrifuged for 5 min at 8000 *g*. Serial dilutions of AOAP in the homogenization buffer were prepared and processed in an identical fashion. Samples of AOAP solution or of the clear supernatant from the boiled extract were assayed for their ability to inhibit mouse kidney ODC (Seely *et al.*, 1982). The content of AOAP in the cell extracts was then estimated by comparison of the inhibition by these extracts with that produced by the standard AOAP solution. Although the standard AOAP solution was dissolved in the same buffer and heat-treated in the same way as the cell extracts, control experiments showed that this treatment had no effect on the ability of the compound to inhibit ODC.

RESULTS

As shown in Fig. 1, AOAP was a potent inhibitor of cell growth in L1210 cells. As little as 20 μM -AOAP caused a 55% decrease in cell density after a 72 h incubation period, and growth inhibition was maximal at concentrations of AOAP greater than 50 μM . On the other hand, DFMO-resistant (D-R) variant cells derived from the L1210 cell line (Pegg *et al.*, 1988a) were completely unaffected by AOAP concentrations up to 0.5 mM (Fig. 1). Growth rates in the two cell lines did not differ significantly in the absence of the drug. Only at 1 mM-AOAP could any significant inhibition (~30%) of D-R cell proliferation be detected, but this effect was transient and cell density was restored to control levels after 78 h (results not shown). No attempt was made to examine whether the modest effect produced by 1 mM-AOAP on growth was reversible by addition of

Fig. 1. Effect of AOAP on growth of L1210 and D-R cells

Both cell lines were initially seeded at 5×10^4 cells/ml, and AOAP was then added to the cell cultures at the concentration shown. Samples of the suspensions were taken for cell counting 72 h after drug addition. Results for L1210 (●) and D-R (○) cells are expressed as means $\pm$ S.D.

Table 1. Effect of AOAP and polyamines on growth of L1210 and D-R cells

Cell number was determined at the time indicated after addition of the drug, which was done at the time of cell seeding. The initial density was set at 5×10^4 cells/ml for both cell lines. Results are given as means $\pm$ S.D.

Treatment	Time (h)	$10^{-6} \times$ Cell no./ml	
		L1210 cells	D-R cells
None	24	0.17 ± 0.01	0.19 ± 0.01
	48	0.77 ± 0.05	0.75 ± 0.05
	72	1.23 ± 0.06	1.32 ± 0.05
+ 50 μ M-Putrescine	24	0.18 ± 0.02	0.19 ± 0.01
	48	0.74 ± 0.05	0.74 ± 0.07
	72	1.32 ± 0.08	1.25 ± 0.07
+ 10 μ M-Spermidine	24	0.18 ± 0.01	0.18 ± 0.02
	48	0.76 ± 0.03	0.75 ± 0.07
	72	1.30 ± 0.09	1.25 ± 0.05
+ 100 μ M-AOAP	24	0.12 ± 0.01	0.19 ± 0.01
	48	0.42 ± 0.01	0.76 ± 0.04
	72	0.42 ± 0.02	1.23 ± 0.11
+ 100 μ M-AOAP + 50 μ M-putrescine	24	0.19 ± 0.01	0.18 ± 0.01
	48	0.76 ± 0.05	0.79 ± 0.07
	72	1.29 ± 0.08	1.27 ± 0.06
+ 100 μ M-AOAP + 10 μ M-spermidine	24	0.18 ± 0.01	0.19 ± 0.01
	48	0.71 ± 0.03	0.81 ± 0.03
	72	1.23 ± 0.04	1.31 ± 0.04
+ 5 mM-DFMO	24	0.13 ± 0.01	0.18 ± 0.02
	48	0.37 ± 0.02	0.79 ± 0.08
	72	0.35 ± 0.02	1.33 ± 0.07

polyamines, because the decrease was only apparent over a short time period.

Although growth was essentially arrested 48 h after the addition of 100 μ M-AOAP, the concomitant addition of either putrescine (50 μ M) or spermidine (10 μ M) completely reversed the AOAP-induced inhibition of cell proliferation in L1210 cells (Table 1). Neither polyamine had any effect alone on growth. The ability of AOAP to inhibit growth in L1210 cells, but not in D-R cells, in conjunction with the reversal of this effect by the further addition of either putrescine or spermidine, suggests that AOAP acts primarily as an inhibitor of ODC in these cells. As shown in Table 1, DFMO, an enzyme-activated irreversible inhibitor of ODC, inhibited L1210-cell growth to virtually the same degree than AOAP. As expected (Pegg *et al.*, 1988a), D-R cell growth was unresponsive to the addition of DFMO.

Since AOAP is a potential inhibitor of both ODC and AdoMetDC (Khomutov *et al.*, 1985a; Hyvönen *et al.*, 1988), the effect of AOAP on the activities of both enzymes was examined 48 and 72 h after drug addition to L1210 and D-R cells (Table 2). When assayed in undialysed extracts, there was clearly a substantial decrease in ODC activity in both cell lines. Little, if any, detectable ODC activity was found in crude extracts from L1210 cells which had been grown in the presence of AOAP. However, dialysis of these extracts unmasked a substantial increase in ODC activity produced by drug treatment, especially at 72 h (up to 11-fold). Higher (≥ 500 μ M) concentrations of AOAP were significantly more effective in inducing an increase in ODC activity, indicating a dose-dependent effect of AOAP on this parameter. Undialysed extracts from the AOAP-treated

Table 2. Effect of incubation with AOAP on ODC and AdoMetDC activities in L1210 and D-R cells

Both control and D-R cells were suspended in fresh medium at 10^5 cells/ml, and AOAP was added at the indicated concentration. Extracts were prepared as described in the Materials and methods section and assayed for enzyme activity with or without dialysis against the appropriate homogenization buffer as indicated. Values are given as means $\pm$ S.D.

[AOAP] (mM)	Time (h)	Dialysis	Activity (units/mg)			
			ODC		AdoMetDC	
			L1210	D-R	L1210	D-R
0	48	+	24.2 ± 3.7	2301 ± 611	9.3 ± 1.4	7.2 ± 1.2
	48	—	20.1 ± 1.6	2788 ± 305	11.1 ± 1.6	6.6 ± 0.9
	72	+	5.2 ± 1.5	929 ± 144	4.5 ± 0.7	2.9 ± 0.9
	72	—	4.3 ± 0.7	711 ± 80	6.0 ± 0.6	4.0 ± 0.4
0.2	48	+	57.4 ± 8.2	3526 ± 803	15.9 ± 2.3	7.2 ± 1.3
	48	—	2.1 ± 0.6	366 ± 28	17.5 ± 2.0	8.0 ± 1.1
	72	+	26.1 ± 1.1	3018 ± 612	19.2 ± 3.2	14.1 ± 3.0
	72	—	3.8 ± 1.3	1082 ± 99	23.7 ± 1.9	11.8 ± 0.9
0.5	48	+	83.3 ± 10.5	4075 ± 519	32.3 ± 5.5	3.2 ± 1.0
	48	—	< 0.1	233 ± 42	28.2 ± 3.4	3.7 ± 0.5
	72	+	41.3 ± 4.4	2855 ± 367	15.7 ± 3.2	18.8 ± 2.9
	72	—	3.6 ± 0.6	1799 ± 248	18.3 ± 1.0	17.3 ± 2.1
1.0	48	+	76.6 ± 3.3	480 ± 29	38.4 ± 6.7	2.2 ± 0.5
	48	—	< 0.1	36 ± 4	32.9 ± 2.7	2.8 ± 0.3
	72	+	43.7 ± 2.8	2111 ± 200	16.3 ± 2.4	9.3 ± 2.4
	72	—	3.9 ± 0.8	112 ± 20	19.1 ± 2.5	7.1 ± 0.8

Fig. 2. Inhibition of mouse kidney ODC by extracts from AOAP-treated cells

Extracts were prepared from L1210 cells (○) and from D-R cells (●) grown for 48 h in the presence of 0.2 mM-AOAP and tested for the ability to inhibit mouse kidney ODC as shown. Extracts from control cells not exposed to the drug prepared in the same way had no effect on the ODC activity even when extracts corresponding to 0.25 mg of protein were used. The mouse kidney ODC used was equivalent to 11.5 units when assayed in the absence of inhibitor.

D-R cells did contain some residual ODC activity, although at 48 h this was less than 10% of the activity in the D-R cells not exposed to the drug. However, it should be noted that these extracts were assayed at a greater dilution than the extracts from L1210 cells, because the basal ODC activity was so much higher. When assayed at similar dilutions, the decrease in ODC activity in the D-R cells was 99.7% at 48 h and 98.2% at 72 h, whereas in the L1210 cells the decrease was > 99% and 89% respectively. There was a small increase in ODC activity in the D-R cells in response to AOAP when the extracts were freed from the drug by dialysis. This increase was smaller than in the L1210 cells, but the D-R cells already contain very high levels of ODC, amounting to 1 part in 300 of the soluble protein (Pegg *et al.*, 1988a), and major increases in this value may not be possible.

The activity of AdoMetDC was increased in a dose-dependent manner up to 4-fold in L1210 cells after incubation with AOAP. Dialysis did not affect activity measurements significantly (Table 2). In D-R cells, treatment with higher levels of AOAP did result in a 60–70% decrease in AdoMetDC activity at 48 h, but this was followed by a marked increase after 72 h. The initial decrease in AdoMetDC levels induced by AOAP required higher concentrations ($\geq 500 \mu\text{M}$) than the subsequent increase, suggesting that different molecular targets of the AOAP were responsible for the two diverging effects of the drug.

The uptake of AOAP into the L1210 and the D-R cells was determined by measuring the ability of extracts of the cells which had been grown for 48 h in the presence of 0.2 mM-AOAP to inhibit the activity of mouse kidney ODC. As shown in Fig. 2, both extracts were potent inhibitors of the ODC activity, and there was no significant difference in the content between the two cell lines. An estimate of the AOAP concentration present in

Fig. 3. Inhibition of mouse kidney ODC by AOAP

Mouse kidney ODC (11.5 units) was assayed in the presence of portions of an AOAP solution prepared in the same way as the extracts used in Fig. 2. The percentage of activity found in the presence of the AOAP concentration indicated was then calculated as shown. Results are shown as means $\pm$ S.D. Where no error bar is shown, the variation was less than the size of the symbol.

the cells was obtained by comparing the inhibition with that produced by a solution of AOAP treated in exactly the same way (Fig. 3). This indicated that the content of the L1210 cells corresponded to 41 ± 6 pmol/mg of protein and that of the D-R cells was 41 ± 4 pmol/mg of protein. These values correspond to an intracellular concentration of about $0.5 \mu\text{M}$.

The effect of AOAP on the intracellular contents of polyamines and decarboxylated AdoMet after a 72 h incubation period in control and D-R L1210 cells is illustrated in Fig. 4. In L1210 cells, putrescine contents were decreased to undetectable levels at the lowest AOAP concentration tested ($20 \mu\text{M}$) (Fig. 4a). In contrast, AOAP caused a dose-dependent decrease in putrescine contents over the whole range of concentrations tested in D-R cells, which had about 8-fold higher putrescine levels than L1210 cells under basal conditions. Putrescine was still detectable in D-R cells incubated with $500 \mu\text{M}$ -AOAP (Fig. 4a). Spermidine content was decreased to extremely low levels by AOAP treatment in L1210 cells (Fig. 4b). Although further depletion of intracellular spermidine was induced by increasing AOAP concentrations from 20 to $200 \mu\text{M}$ in these cells, a 97% decrease in the spermidine pool was already effected by $20 \mu\text{M}$ -AOAP. On the other hand, AOAP was effective in decreasing spermidine contents only at concentrations $\geq 200 \mu\text{M}$ in the D-R cells, although spermidine levels did not significantly differ between the two cell lines in the absence of the drug. There was an approx. 25% increase in spermine content in L1210 cells incubated with concentrations of AOAP $\geq 20 \mu\text{M}$ (Fig. 4c). The drug increased spermine levels to the same absolute value in the D-R line, but this effect required at least $100 \mu\text{M}$ -AOAP to become significant, and basal contents were about 40% lower than in L1210 cells.

Under basal conditions, decarboxylated AdoMet could be detected in L1210 cells, but not in their DFMO-resistant counterparts (Fig. 4d). AOAP induced a striking accumulation of decarboxylated AdoMet in L1210 cells, which was maximal at drug concentrations of $50 \mu\text{M}$ or

Fig. 4. Effect of AOAP on intracellular contents of polyamines and decarboxylated AdoMet in L1210 and D-R cells

Cells were treated as in Fig. 1, and at 72 h cultures were harvested for measurement of putrescine (a), spermidine (b), spermine (c) or decarboxylated AdoMet (d). Results for L1210 (○) and D-R (●) cells are expressed as means $\pm$ S.D.

Table 3. Effect of AOAP and polyamines on intracellular contents of polyamines and decarboxylated AdoMet in L1210 and D-R cells

Both cell lines were seeded at 5×10^4 cells/ml, and all additions were made at the time of seeding. Cells were harvested for measurements 72 h after the addition shown. Results are means $\pm$ S.D.

Treatment	Cells	Polyamine content (nmol/mg of protein)			
		Putrescine	Spermidine	Spermine	Decarboxylated AdoMet
No addition	L1210	4.5 ± 0.2	32.5 ± 6.5	12.7 ± 2.3	0.33 ± 0.02
	D-R	36.3 ± 1.5	40.1 ± 1.5	7.2 ± 0.2	< 0.025
50 μ M-Putrescine	L1210	4.9 ± 0.3	31.8 ± 3.0	9.4 ± 1.5	0.19 ± 0.04
	D-R	58.4 ± 6.1	40.9 ± 5.9	8.8 ± 1.3	< 0.025
10 μ M-Spermidine	L1210	0.28 ± 0.08	36.8 ± 0.6	17.4 ± 1.8	0.24 ± 0.04
	D-R	25.7 ± 2.2	60.3 ± 3.6	10.9 ± 1.8	< 0.025
100 μ M-AOAP	L1210	< 0.1	0.26 ± 0.01	17.5 ± 3.5	3.75 ± 0.23
	D-R	9.7 ± 2.3	38.5 ± 3.1	11.9 ± 2.4	< 0.025
100 μ M-AOAP + 50 μ M-putrescine	L1210	5.3 ± 0.5	41.1 ± 0.2	9.9 ± 0.4	< 0.025
	D-R	26.3 ± 2.8	39.9 ± 4.2	7.7 ± 0.7	< 0.025
100 μ M-AOAP + 100 μ M-spermidine	L1210	0.14 ± 0.01	42.1 ± 0.4	12.6 ± 2.1	< 0.025
	D-R	7.9 ± 1.7	43.1 ± 3.0	9.8 ± 1.1	< 0.025
5 mM-DFMO	L1210	< 0.1	< 0.1	18.0 ± 0.9	3.26 ± 0.20
	D-R	0.17 ± 0.01	10.9 ± 1.2	11.1 ± 1.1	0.38 ± 0.03

above. A much smaller accumulation of decarboxylated AdoMet was seen in the D-R cells and, as with polyamines, significant changes required much higher concentrations ($\geq 200 \mu$ M) of the inhibitor than in L1210 cells (Fig. 4d).

The addition of 50 μ M-putrescine or 10 μ M-spermidine restored spermidine and spermine contents to near con-

trol levels in both cell lines exposed to 100 μ M-AOAP (Table 3). Exogenous spermidine (10 μ M) decreased putrescine levels in the absence of the drug, as expected from its known inhibitory effect on ODC levels (Pegg, 1986; Porter *et al.*, 1987a), and had little effect on this parameter in AOAP-treated cells, indicating that ODC inhibition by AOAP was probably already maximal

(Table 3). Addition of either putrescine or spermidine to L1210 cells treated with 100 μ M-AOAP not only blocked the AOAP-induced accumulation of decarboxylated AdoMet but also further decreased the intracellular content of this nucleoside to undetectable levels. The addition of exogenous putrescine or spermidine alone decreased the intracellular content of decarboxylated AdoMet by 30–40% in L1210 cells (Table 3).

DISCUSSION

It appears that AOAP inhibits polyamine metabolism and growth in L1210 cells by decreasing the activity of ODC. The drug led to a substantial decrease in ODC activity when the activity was assayed in undialysed extracts from treated cells. The ODC activity in both L1210 and D-R cells was decreased by more than 90%, but there was sufficient residual ODC activity in the D-R cells to maintain levels of polyamines sufficient for growth because of the much higher initial ODC content in the D-R cells. The changes in intracellular polyamine levels brought about by AOAP in L1210 cells are similar to those produced by the well-known ODC inhibitor DFMO in many cell types, with virtually complete depletion of putrescine and spermidine, a small increase in spermine and a large increase in decarboxylated AdoMet (reviewed by Pegg, 1986; Porter & Jänne, 1987; Danzin & Mamont, 1987). The absence of inhibitory effects on the growth of D-R cells, which have a large excess of ODC (Pegg *et al.*, 1988a), is also consistent with this interpretation. AOAP does not appear to be an effective inhibitor of AdoMetDC. The activity of this enzyme was not decreased in L1210 cells treated with AOAP, and there was no decline in spermine, which occurs with authentic inhibitors of this enzyme such as 5'-deoxy-5'-[N-methyl-N-[2-(amino-oxy)ethyl]]amino-adenosine ('MAOEA') and 5'-deoxy-5'-[N-methyl-N-(3-hydrazinopropyl)]aminoadenosine ('MHZPA') (Pegg *et al.*, 1988b). Furthermore, the growth of D-R cells is known to be slowed when AdoMetDC activity is blocked by these compounds (Pegg *et al.*, 1988a). The small decrease in AdoMetDC at the earlier time points in D-R cells treated with high concentrations of AOAP may reflect a small degree of inhibition which at later times is masked by the compensatory increase in response to spermidine depletion (see below).

Our results provide further support to the conclusion reached from comparisons of the polyamine levels in L1210 and D-R cells, that the production of putrescine is not the limiting factor in the synthesis of spermidine and spermine in these cells (Pegg *et al.*, 1988a). Although ODC activity and putrescine content in the D-R cells were decreased in a dose-dependent manner by AOAP over the entire range of 20–200 μ M, there was little effect on spermidine or spermine levels until AOAP concentrations of more than 100 μ M were reached.

The lack of effect of AOAP at concentrations of up to 0.5 mM on growth of D-R cells is unlikely to be due to decreased uptake or increased excretion of the drug, since (a) both ODC activity and putrescine content are decreased by exposure to AOAP (Table 2 and Fig. 4) and (b) extracts from the AOAP-treated D-R cells have the same inhibitory activity towards purified ODC as similar extracts from L1210 cells (Fig. 3).

Our findings of an increased activity of both AdoMetDC and ODC (after dialysis) in extracts from

L1210 and D-R cells treated with AOAP is in agreement with the findings by Hyvönen *et al.* (1988) on BHK cells and by Persson *et al.* (1989) on Ehrlich ascites cells. It is highly probable that these increases are due to the decline in cellular spermidine levels, since compensatory increases in both enzymes have been observed in experiments when spermidine is depleted with other inhibitors (Alhonen-Hongisto, 1980; Mamont *et al.*, 1981; Hölttä & Pohjanpelto, 1986; Persson *et al.*, 1986; Dirks *et al.*, 1986; McConlogue *et al.*, 1986; Porter *et al.*, 1987a,b, 1988; Madhubala *et al.*, 1988). These compensatory changes tend to reverse the effect of inhibitors, but the AOAP is clearly effective at blocking putrescine production at quite low concentrations even though its activity on ODC is reversible. In fact, AOAP prevents putrescine production in L1210 cells at concentrations lower than DFMO, and is comparable in this respect with some of the more potent irreversible inactivators of ODC such as (2R,5R)hept-6-yne-2,5-diamine (Mamont *et al.*, 1984) and the methyl ester of E-(2-fluoromethyl)dehydro-orithine (Mamont *et al.*, 1986). The specificity of AOAP towards ODC is indicated by its lack of effect on the growth of D-R cells and by the ready reversal of its growth inhibitory properties by provision of exogenous spermidine or putrescine. The possibility that AOAP might be a useful pharmacological agent in some systems by acting to prevent polyamine synthesis at the ODC step merits further study.

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