Biochimica et Biophysica Acta, 972 (1988) 239–248
Elsevier

BBA 12348

Feedback regulation of polyamine synthesis in Ehrlich ascites tumor cells. Analysis using nonmetabolizable derivatives of putrescine and spermine

Ingvar Holm [a], Lo Persson [b], Olle Heby [a] and Nikolaus Seiler [c]

Departments of [a] Zoophysiology and [b] Physiology, University of Lund, Lund (Sweden) and [c] Merrell Dow Research Institute, Strasbourg Center, Strasbourg (France)

(Received 11 April 1988)
(Revised manuscript received 1 August 1988)

Key words: Polyamine; Ornithine decarboxylase; *S*-Adenosylmethionine decarboxylase; Translational control; (Ehrlich ascites tumor cell)

Ornithine decarboxylase (ODC) is subject to feedback regulation by the polyamines. Thus, addition of putrescine, spermidine or spermine to cells causes inhibition of ODC mRNA translation. Putrescine and spermine are readily converted into spermidine. Therefore, it is conceivable that the inhibition of ODC synthesis observed in putrescine- and spermine-supplemented cells is instead an effect of spermidine. To examine this possibility we have used two analogs of putrescine and spermine, namely 1,4-dimethylputrescine and 5,8-dimethylspermine, which cannot be converted into spermidine. Both analogs were found to inhibit the incorporation of [^{35}S]methionine into ODC protein to approximately the same extent, suggesting that putrescine as well as spermine exert a negative feedback control of ODC mRNA translation in the cell. In addition to suppressing ODC synthesis, both analogs were found to increase the turnover rate of the enzyme. 5,8-Dimethylspermine caused a marked decrease in the activity of *S*-adenosylmethionine decarboxylase (AdoMetDC). This effect was not obtained with 1,4-dimethylputrescine, indicating that spermine, but not putrescine, exerts a negative control of AdoMetDC. Treatment with 1,4-dimethylputrescine caused extensive depletion of the cellular putrescine and spermidine content, but accumulation of spermine. 5,8-Dimethylspermine treatment, on the other hand, effectively depleted the spermine content and had less effect on the putrescine and spermidine content, at least initially. Nevertheless, the total polyamine content was more extensively reduced by treatment with 5,8-dimethylspermine than with 1,4-dimethylputrescine. Accordingly, only 5,8-dimethylspermine treatment exerted a significant inhibitory effect on Ehrlich ascites tumor cell growth.

Introduction

Specific inactivation of ornithine decarboxylase (ODC) by treatment with various substrate and product analogs has been found to deplete the putrescine and spermidine content rapidly and, as a consequence, to slow the growth and proliferation of many different cell types [1,2]. But even

Abbreviations: ODC, ornithine decarboxylase (EC 4.1.1.17); AdoMetDC, *S*-adenosylmethionine decarboxylase (EC 4.1.1.50); DFMO, D,L-2-difluoromethylornithine; SAT, spermidine/spermine N^1-acetyltransferase; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Correspondence: O. Heby, Department of Zoophysiology, University of Lund, S-22362 Lund, Sweden.

the natural polyamines putrescine, spermidine and spermine decrease the activity of ODC when added to cells in culture [1,3–5]. There is evidence to suggest that part of this effect is due to post-translational regulation. Thus, treatment with polyamines may induce the synthesis and/or release of an ODC-inhibitory protein named antizyme [6–10].

In recent studies, evidence has been obtained for polyamine-mediated regulation of ODC expression at the translational level [11–15]. Addition of putrescine, spermidine or spermine to cells decreases the efficiency by which ODC mRNA is translated. On the other hand, if cells are depleted of polyamines by treatment with 2-difluoromethylornithine (DFMO) an ODC inhibitor, the translation of ODC mRNA is increased [12]. DFMO treatment decreases the cellular putrescine and spermidine content, whereas the spermine content is unaffected or even slightly increased.

Putrescine and spermine are readily converted into spermidine in the cells [16–18]. Hence, it is conceivable that the observed ODC-regulatory effects of putrescine and spermine are mediated by spermidine. To study this possibility we have used a putrescine and a spermine analog, 1,4-dimethylputrescine [19] and 5,8-dimethylspermine. These analogs (Fig. 1) cannot be converted into spermidine, because the methyl groups make them unsuitable as substrates for spermidine synthase and spermidine/spermine N^1-acetyltransferase (SAT),

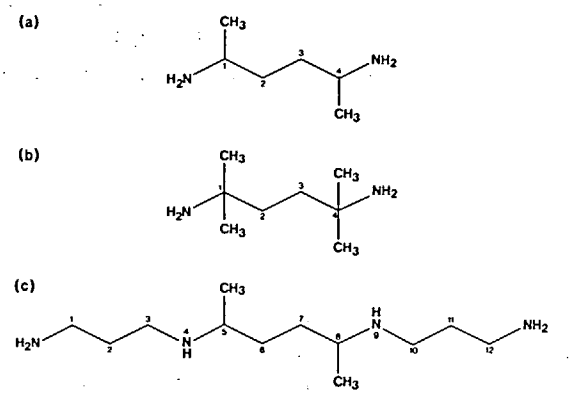

Fig. 1. Structural formulas of methyl-substituted polyamine analogs. (a) 1,4-Dimethylputrescine; (b) 1,1,4,4-tetramethylputrescine; (c) 5,8-dimethylspermine.

due to steric hindrance (Ref. 19 and F.N. Bolkenius, unpublished observations).

We also address the question whether these analogs, by affecting polyamine-metabolizing enzymes, cause depletion of endogenous polyamines and, as a consequence, inhibit cell proliferation. Both 1,4-dimethylputrescine and 5,8-dimethylspermine are shown to inhibit ODC synthesis and to increase the turnover rate of ODC. In addition, 5,8-dimethylspermine reduces the *S*-adenosylmethionine decarboxylase (AdoMetDC) activity and is more effective than 1,4-dimethylputrescine in depleting the endogenous polyamine content. Accordingly 5,8-dimethylspermine, but not 1,4-dimethylputrescine, is found to possess a significant anti-proliferative action.

Materials and Methods

Chemicals. Cell culture media were purchased from NordVacc (Stockholm, Sweden). L-1-[^{14}C]Ornithine (spec. act. 54.3 mCi/mmol) and [*acetyl*-1-^{14}C]acetyl-CoA (spec. act. 57.2 mCi/mmol) were from New England Nuclear (Dreieich, F.R.G.). *S*-Adenosyl-L-[*carboxyl*-^{14}C]methionine (spec. act. 59.5 mCi/mmol) and [^{35}S]methionine (spec. act. > 1000 Ci/mmol) were from Amersham International (Amersham, U.K.). Dithiothreitol, pyridoxal 5′-phosphate, L-ornithine and *S*-adenosyl-L-methionine were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 1,1,4,4-Tetramethylputrescine (base) was from Aldrich (Beerse, Belgium). The dihydrochloride was prepared according to a known procedure. All other commercial chemicals were from E. Merck (Darmstadt, F.R.G.).

DL-2-Difluoromethylornithine · HCl (Eflornithine™; MDL 71,782; DFMO) [20] and 1,4-dimethylputrescine · 2HCl (2,5-hexanediamine · 2HCl) [19] were synthesized according to published methods. 5,8-Dimethylspermine · 4HCl was obtained by reaction of 1,4-dimethylputrescine with two equivalents of acrylonitrile, and catalytic hydrogenation of the formed *N*,*N*′-bis-cyanoethyl-2,5-hexanediamine, using the reaction conditions that were previously described for the preparation of N^8-acetylspermidine [21]. The reaction product was purified by chromatography on a column of Dowex 50-WX8, using an HCl gradient

from 0 to 4.5 M. 5,8-Dimethylspermine · 4HCl was uniform in all chromatographic systems tested and gave the correct elemental analysis. The mass spectrum (chemical ionization mode using ammonia as reagent gas) showed the expected $(M+1)^+$ at 231 and fragment ions at 216, 174 and 155. The proton NMR-spectrum was compatible with the structure of 5,8-dimethylspermine.

Cell line. Suspension cultures of Ehrlich ascites tumor cells were grown in 75 cm^2 Nunc flasks and were incubated without agitation at 37 °C in a water-saturated atmosphere of 5% CO_2 in air. The growth medium used was a 1 : 1 mixture of Eagle's minimum essential medium and Ham's F-12 medium (without putrescine) supplemented with 0.2% bovine serum albumin (fraction V), penicillin (50 IU/ml) and streptomycin (50 μg/ml). The cells were subcultured every 3–4 days by a 20-fold dilution with fresh growth medium.

Cell growth. In each experiment, plateau-phase cells were seeded at a density of $1.0 \cdot 10^5$ cells/ml in the absence or presence of 1 mM 1,4-dimethylputrescine, 1 mM 1,1,4,4-tetramethylputrescine or 0.5 mM 5,8-dimethylspermine. Aliquots were removed 1, 2, 3 and 4 days after seeding and the number of cells in each culture was estimated by counting in a hemocytometer.

Analysis of ODC and AdoMetDC activities. The enzyme activities were determined in cells that had been stored at −70 °C. The cells were sonicated in a small volume (roughly $5 \cdot 10^6$ cells in 500–1000 μl) of ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 0.1 mM EDTA and 0.5 mM dithiothreitol. After centrifugation at $20000 \times g$ for 20 min, ODC and AdoMetDC activities were determined in aliquots of the supernatants by measuring the release of $^{14}CO_2$ from carboxyl-labeled L-ornithine and *S*-adenosyl-L-methionine, respectively.

The reaction mixture used in the ODC activity assay contained saturating levels of pyridoxal 5′-phosphate (0.1 mM) and L-ornithine (0.5 mM) [22]. The final spec. act. of L-[1-^{14}C]ornithine was 2.0 mCi/mmol.

In the AdoMetDC assay the reaction mixture contained 2.5 mM putrescine and 0.2 mM *S*-adenosyl-L-methionine [23]. The final spec. act. of *S*-adenosyl-L-[*carboxyl*-^{14}C]methionine was 0.4 mCi/mmol.

Analysis of ODC synthesis. The synthesis of ODC was determined by measuring the incorporation of L-[^{35}S]methionine into the enzyme as previously described [13,15]. Cells were seeded at a density of $1.0 \cdot 10^6$ cells/ml in methionine-free growth medium. After 10 min preincubation at 37 °C, L-[^{35}S]methionine (10 μCi/ml) was added. Incorporation of label into protein was terminated after 25 min by chasing with L-methionine and cooling the cultures on ice. Cell extracts were incubated with an excess of ODC antiserum [24] and the resulting protein-antibody complexes were precipitated with protein A adsorbent. The immunoreactive material was fractionated by SDS-PAGE essentially as described by Persson et al. [25]. The gels were incubated in Amplify (Amersham International) prior to fluorography.

Analysis of ODC turnover. Cells were grown in the absence or presence of the methyl-substituted polyamine analogs as described above. Cycloheximide (50 μg/ml) was added 1 day after seeding and the cells were analyzed for ODC activity at 30-min intervals.

Polyamine determination. $HClO_4$ extracts of cells were analyzed for their polyamine content using a Varian VISTA 5500 high-performance liquid chromatographic (HPLC) system. The method was essentially the same as that previously described [26]. The polyamines were separated on a reversed-phase column (Beckman Ultrasphere I.P.) after formation of ion pairs with 1-octanesulfonate. *o*-Phthaldialdehyde/2-mercaptoethanol reagent was used for post-column derivatization and fluorescence was recorded at 455 nm (excitation wavelength 345 nm).

Analysis of SAT activity. The activity of SAT was determined by measuring the acetylation of spermidine using radiolabeled acetyl-CoA essentially as described by Persson and Pegg [27].

Results

Effects of methyl-substituted polyamine analogs on ODC and AdoMetDC activities

When Ehrlich ascites tumor cell cultures were diluted with fresh growth medium their ODC and AdoMetDC activities increased dramatically and reached peak levels by day 1–2, i.e., at the time of most rapid growth. Fig. 2 shows the effects of the

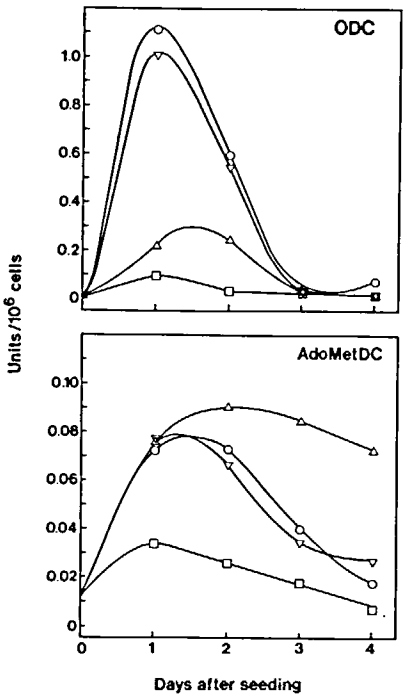

Fig. 2. Effects of methyl-substituted polyamine analogs on the activities of ODC and AdoMetDC in Ehrlich ascites tumor cells. 1 Unit = 1 nmol CO_2 formed per h. Cells were seeded in the absence (○) or presence of 1 mM 1,4-dimethylputrescine (△), 1 mM 1,1,4,4-tetramethylputrescine (▽) or 0.5 mM 5,8-dimethylspermine (□). Values are means of three experiments.

three polyamine analogs on the cellular ODC and AdoMetDC activities. Treatment with 1,1,4,4-tetramethylputrescine (1 mM) had no significant effect on the increases in ODC and AdoMetDC activities. 1,4-Dimethylputrescine (1 mM), however, exerted a pronounced inhibitory effect on the ODC activity. The initial increase in AdoMetDC activity was unaffected by 1,4-dimethylputrescine. At variance with control cells, 1,4-dimethylputrescine-treated cells maintained an elevated AdoMetDC activity throughout growth.

Treatment with 5,8-dimethylspermine (0.5 mM) almost completely prevented the increase in ODC activity (Fig. 2). Moreover, this treatment caused marked suppression of the AdoMetDC activity.

Effects of methyl-substituted polyamine analogs on the cellular polyamine content

As a result of the increased ODC and AdoMetDC activities, induced by dilution with fresh growth medium, the cellular polyamine content increased markedly (Fig. 3). The polyamines reached their peak levels consecutively; putrescine by day 1–2, spermidine by day 2–3 and spermine by day 3.

Fig. 3 shows the effects of the polyamine analogs on the cellular polyamine content. Treatment

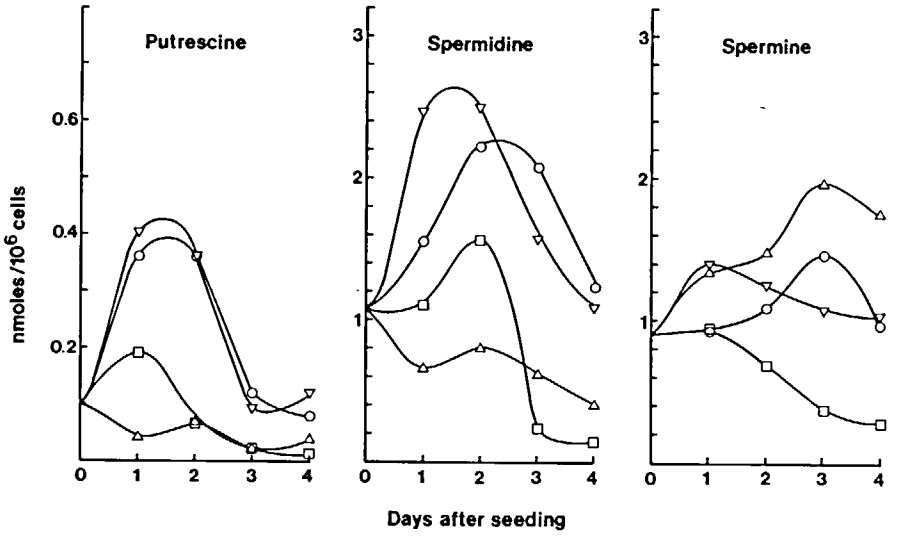

Fig. 3. Effects of methyl-substituted polyamine analogs on the putrescine, spermidine and spermine content of Ehrlich ascites tumor cells. Cells were seeded in the absence (○) or presence of 1 mM 1,4-dimethylputrescine (△), 1 mM 1,1,4,4-tetramethylputrescine (▽) or 0.5 mM 5,8-dimethylspermine (□). Values are means of three experiments.

with 1,1,4,4-tetramethylputrescine (1 mM) had no significant effect on the increase in putrescine content. Even though treatment with this analog did not prevent the increases in spermidine and spermine content, it caused a shift of both peaks to earlier time points.

Treatment with 1,4-dimethylputrescine (1 mM) not only prevented the increases in putrescine and spermidine content, but markedly reduced their basal levels (Fig. 3). The spermine content, on the other hand, increased more rapidly and to a higher level in 1,4-dimethylputrescine-treated cells than in untreated control cells.

The effects of 5,8-dimethylspermine (0.5 mM) on the cellular putrescine and spermidine levels were initially less pronounced than those of 1,4-dimethylputrescine (Fig. 3). However, toward the end of the experiment (day 3–4), 5,8-dimethylspermine-treated cells were more extensively depleted of putrescine and spermidine than were their 1,4-dimethylputrescine-treated counterparts.

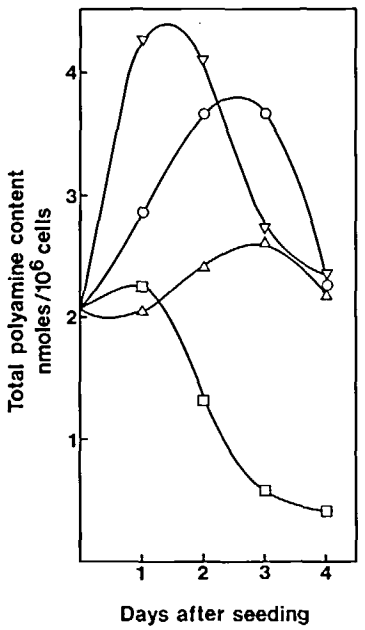

Fig. 4. Effects of methyl-substituted polyamine analogs on the total endogenous polyamine content of Ehrlich ascites tumor cells. The individual values for putrescine, spermidine and spermine (Fig. 3) were added. Cells were seeded in the absence (○) or presence of 1 mM 1,4-dimethylputrescine (△), 1 mM 1,1,4,4-tetramethylputrescine (▽) or 0.5 mM 5,8-dimethylspermine (□). Values are means of three experiments.

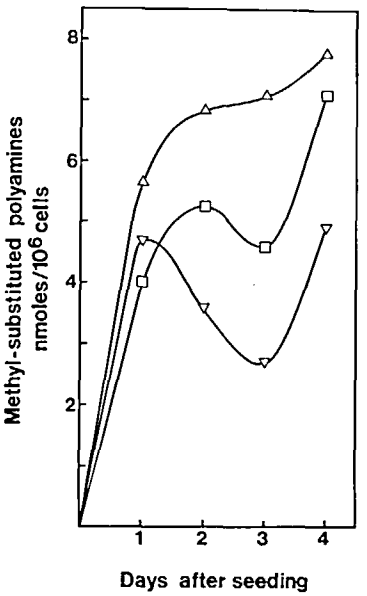

Fig. 5. Accumulation of methyl-substituted polyamine analogs in Ehrlich ascites tumor cells. Cells were seeded in the presence of 1 mM 1,4-dimethylputrescine (△), 1 mM 1,1,4,4-tetramethylputrescine (▽) or 0.5 mM 5,8-dimethylspermine (□). Values are means of three experiments.

In addition, 5,8-dimethylspermine treatment caused marked depletion of the cellular spermine content.

Although 1,4-dimethylputrescine was able to prevent the normally occurring increase in total polyamine content, 5,8-dimethylspermine was the only polyamine analog of those tested that reduced the total cellular polyamine content markedly below the basal level (Fig. 4).

Cells that were treated with methyl-substituted polyamines accumulated each analog in an amount similar to or exceeding the total endogenous polyamine content (Fig. 5). The most rapid increase in their cellular content occurred during the first day of incubation. It is not known how precisely the observed levels reflect the intracellular content. At least some of these positively charged molecules may be bound to the cell surface.

Effects of methyl-substituted polyamine analogs on ODC synthesis

As seen in Fig. 2, the cellular ODC activity increased dramatically when plateau-phase cultures were diluted with fresh growth medium. The

TABLE I

EFFECTS OF METHYL-SUBSTITUTED POLYAMINES ON THE ACTIVITY OF SAT IN EHRLICH ASCITES TUMOR CELLS

Cells were seeded in the absence (control) or presence of 1 mM 1,4-dimethylputrescine (DMPut) or 0.5 mM 5,8-dimethylspermine (DMSpm). 1 unit of enzyme activity = 1 pmol acetylspermidine formed per min. Means ± S.E. ($n = 3$), except for DMPut, where the values represent averages of two experiments.

Treatment	SAT activity (U/10^6 cells)				
	day 0	day 1	day 2	day 3	day 4
Control	0.62 ± 0.20	0.88 ± 0.27	1.73 ± 0.48	1.87 ± 0.45	0.79 ± 0.07
DMPut	–	1.65	1.67	2.08	0.87
DMSpm	–	2.20 ± 0.84	2.04 ± 0.28	2.43 ± 0.39	1.09 ± 0.31

effects of the polyamine analogs on ODC synthesis were analyzed at the time of peak ODC activity, i.e., 1 day after seeding. The rate of ODC protein synthesis was determined by [^{35}S]methionine pulse-labeling and subsequent SDS-PAGE analysis of immunoprecipitated material (Fig. 6). 1,4-Dimethylputrescine (1 mM) and 5,8-dimethylspermine (0.5 mM), but not 1,1,4,4-tetramethylputrescine (1 mM), exerted marked inhibitory effects on ODC synthesis. Both analogs inhibited ODC synthesis to approximately the same extent. Total protein synthesis was not affected by the methyl-substituted polyamine analogs.

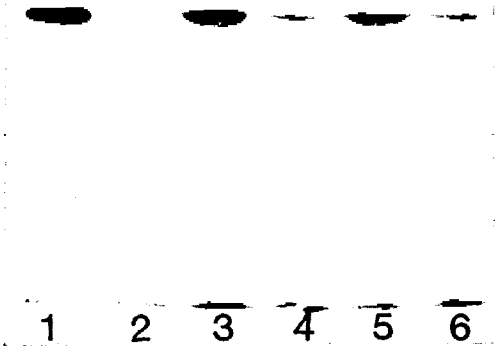

Fig. 6. Effects of methyl-substituted polyamine analogs on ODC synthesis in Ehrlich ascites tumor cells. Extracts from cells labeled with [^{35}S]methionine (after growth for 1 day in the absence or presence of a methyl-substituted polyamine analog) were precipitated with ODC antiserum and analyzed on SDS-PAGE. Lane 1, pure mouse kidney ODC (53 kDa) labeled with [^{3}H]DFMO. Lane 2, untreated control cells, non-immune serum. Lane 3, untreated control cells. Lane 4, cells treated with 1 mM 1,4-dimethylputrescine. Lane 5, cells treated with 1 mM 1,1,4,4-tetramethylputrescine. Lane 6, cells treated with 0.5 mM 5,8-dimethylspermine.

The incorporation of [^{35}S]methionine into ODC protein, as demonstrated in Fig. 6, may be somewhat underestimated (notably in 1,4-dimethylputrescine- and 5,8-dimethylspermine-treated cells) due to the relatively long labeling period (25 min) as compared to the half-life of ODC (15–60 min) (this study, and Ref. 11).

Effects of methyl-substituted polyamine analogs on ODC turnover

When cells were grown in the presence of 1,4-dimethylputrescine (1 mM) or 5,8-dimethylspermine (0.5 mM) there was an increase in the rate of ODC turnover. The ODC half-life was 16 min and 14 min, respectively, as compared to 29 min for that of the control cells. 1,1,4,4-Tetramethylputrescine (1 mM) had no significant effect on the turnover rate of ODC.

The increase in ODC turnover can only partly explain the 1,4-dimethylputrescine- and 5,8-dimethylspermine-mediated decrease in ODC activity. The major part of the decrease in ODC activity must be caused by a reduced rate of synthesis, since the change in half-life was only

2-fold as compared to the 5–12-fold change in activity.

Effects of methyl-substituted polyamine analogs on SAT activity

As shown in Table I, the cellular SAT activity increased when plateau-phase cultures were diluted with fresh growth medium. It reached a peak level by day 2–3, i.e., at the time when spermidine and spermine exhibit their highest cellular levels (Fig. 3). Treatment with 1,4-dimethylputrescine initially caused a slight stimulation of SAT activity, but from day 2 on, the activity did not differ from that of the untreated control (Table I). 5,8-Dimethylspermine treatment caused a somewhat greater stimulation of SAT activity, which tended to exceed that of untreated control cells throughout the experimental growth period.

Effects of methyl-substituted polyamine analogs on cell growth

Ehrlich ascites tumor cells grew normally in a medium supplemented with 1,4-dimethylputrescine (1 mM) or 1,1,4,4-tetramethylputrescine (1 mM). Treatment with 5,8-dimethylspermine (0.5 mM) had no adverse effect on cell growth during the first 2 days, but subsequently reduced the growth rate considerably (Fig. 7).

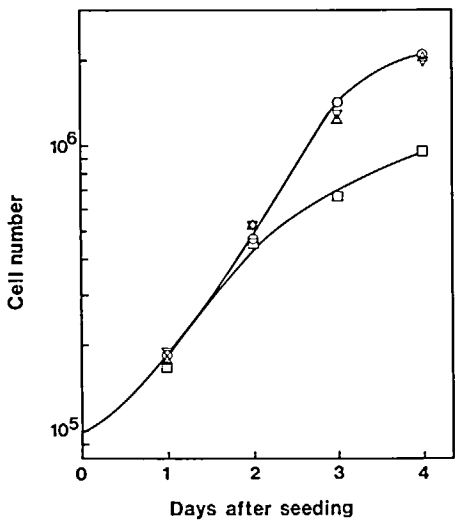

Fig. 7. Effects of methyl-substituted polyamine analogs on Ehrlich ascites tumor cell growth. Cells were seeded in the absence (○) or presence of 1 mM 1,4-dimethylputrescine (△), 1 mM 1,1,4,4-tetramethylputrescine (▽) or 0.5 mM 5,8-dimethylspermine (□). Values are means of three experiments.

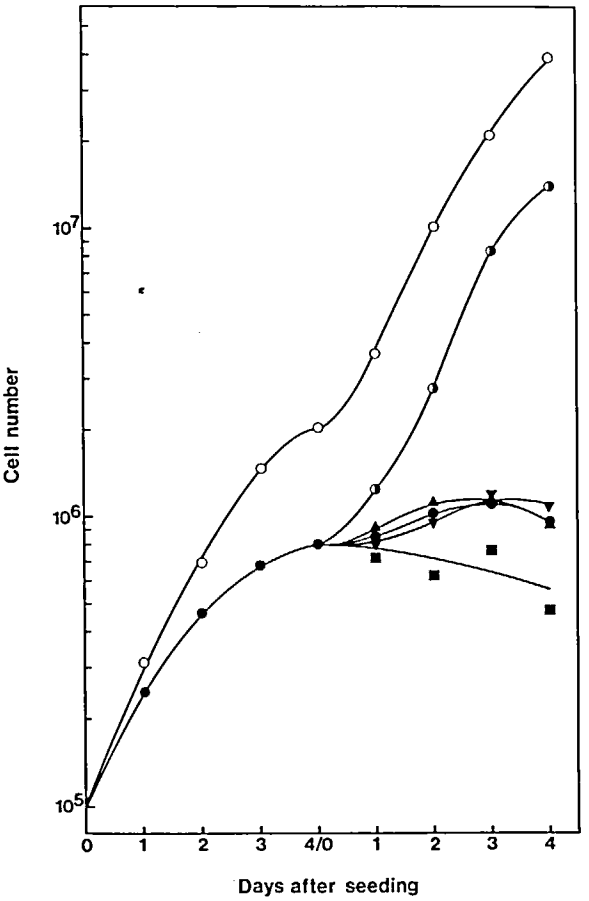

Fig. 8. Analysis of the ability of methyl-substituted polyamine analogs to reverse growth arrest resulting from putrescine and spermidine depletion. Ehrlich ascites tumor cells were seeded in the absence (○) or presence (●) of 5 mM DFMO. DFMO causes depletion of the putrescine and spermidine content [28–30]. Upon growth arrest (day 4) the DFMO-treated cells were supplemented with 1 mM putrescine (◑), 1 mM 1,4-dimethylputrescine (▲), 1 mM 1,1,4,4-tetramethylputrescine (▼) or 0.5 mM 5,8-dimethylspermine (■). Values are means of three experiments.

Effects of methyl-substituted polyamine analogs on cells arrested in their growth due to polyamine depletion

When Ehrlich ascites tumor cells are seeded in the presence of 5 mM DFMO, their putrescine and spermidine content is rapidly depleted [28–30].

Consequently, the growth rate is reduced and the cells eventually cease to grow. Upon addition of putrescine, however, the cells rapidly resume a normal growth rate [30] (Fig. 8). At variance with the results obtained with putrescine, 1,4-dimethylputrescine and 5,8-dimethylspermine were unable to reverse the growth-inhibited state. Likewise, when added to cell cultures at the same time as DFMO, these analogs could not prevent the DFMO-mediated growth arrest (not shown).

Discussion

In eukaryotes, ODC is regulated at transcriptional, translational and post-translational levels (for a review, see Refs. 31 and 32). When cells are blocked in their polyamine synthesis by treatment with inhibitors of ODC, their growth and proliferation cease [28–30]. We recently presented evidence suggesting that the rate of ODC translation is a direct function of the intracellular polyamine level [13,15]. Reduction in cellular putrescine and spermidine content by DFMO treatment causes stimulation of ODC mRNA translation, whereas addition of putrescine, spermidine or spermine causes inhibition [13–15]. Since putrescine and spermine are readily converted into spermidine, the latter polyamine may be the actual regulator of ODC mRNA translation. In fact, ODC mRNA translation in reticulocyte lysates was found to be inhibited to a greater extent by spermidine than by putrescine (Holm, I. et al., unpublished observations).

To investigate this possibility further, we used 1,4-dimethylputrescine and 5,8-dimethylspermine, which cannot be converted into spermidine, since they are poor substrates of spermidine synthase and SAT. The fact that 1,4-dimethylputrescine inhibited the incorporation of [^{35}S]methionine into ODC protein indicates that not only spermidine but also putrescine exerts a negative feedback control of ODC mRNA translation in the cell. We cannot fully exclude the possibility that the adverse effect of 1,4-dimethylputrescine on the ODC activity is mediated through displacement of intracellularly bound spermidine. However, such an increase in free spermidine would also be expected to suppress the AdoMetDC activity [33], which was not the case (Fig. 2).

Whether the spermine structure exerted a negative feedback control of ODC mRNA translation was tested in a similar manner using 5,8-dimethylspermine. Indeed, 5,8-dimethylspermine inhibited the incorporation of [^{35}S]methionine into ODC protein to approximately the same extent as 1,4-dimethylputrescine did.

Our previous studies [13,15] indicate that polyamines regulate ODC expression mainly at the translational level, because the ODC mRNA level remains constant. Accordingly, the C-methylated derivatives of putrescine and spermine were found to down-regulate ODC synthesis without markedly affecting the steady-state level of ODC mRNA (not shown).

In addition to suppressing the ODC activity, 5,8-dimethylspermine treatment effectively counteracted the normally occurring increase in AdoMetDC activity. This effect was not obtained with 1,4-dimethylputrescine. Instead, the AdoMetDC activity increased as in untreated control cells, and then remained at an elevated level. These findings are in agreement with the contention that AdoMetDC is regulated negatively by spermine and positively by putrescine [31,34]. Recent studies indicate that spermine (and spermidine) regulates AdoMetDC both at the transcriptional and the translational level [34–36].

Treatment with 1,4-dimethylputrescine caused extensive depletion of the cellular putrescine and spermidine content, but accumulation of spermine. Apparently, these changes are all due to the decrease in ODC activity, because AdoMetDC (and spermidine synthase) were not significantly affected by 1,4-dimethylputrescine treatment. In fact, the same changes in polyamine content are found in cells treated with the ODC inhibitor DFMO [28–30]. The cells are first depleted of their putrescine content, which becomes limiting in spermidine synthesis. Because of the deficiency in putrescine, spermidine becomes the preferred acceptor of 3-aminopropyl moieties (derived from decarboxylated *S*-adenosylmethionine). As a consequence, spermidine is converted into spermine, thus explaining the concurrent decrease in spermidine and increase in spermine content.

The presence of one methyl group in the vicinity of each terminal amino group of putrescine abolishes the capacity of this diamine to act as

substrate for spermidine synthase, without affecting the ability to regulate ODC synthesis. In the case of 1,1,4,4-tetramethylputrescine, the amino groups are so completely screened by the neighboring methyl groups that even their chemical reactivity (e.g., to form Schiff bases) is grossly reduced. It is for this reason that 1,1,4,4-tetramethylputrescine has lost its putrescine-like characteristics and thus its ability to regulate ODC. The growth-stimulatory properties of the polyamines were shown to be sensitive to the presence of a methyl group in the vicinity of the amino groups, because 1,4-dimethylputrescine and 5,8-dimethylspermine were unable to reverse growth arrest resulting from polyamine depletion.

Porter et al. [37,38] have recently shown that alkylation of the terminal amino groups converts the polyamines to antiproliferative agents. The rank order of effectiveness was N^1N^{12}-bis(ethyl) spermine > N^1N^8-bis(ethyl)spermidine > N^1N^4-bis(ethyl)putrescine. The same order of effectiveness was seen in terms of suppression of ODC activity. Whether this involves translational control, however, has not been fully elucidated thus far.

5,8-Dimethylspermine treatment effectively depleted the spermine content but had less effect on the putrescine and spermidine content than 1,4-dimethylputrescine, at least initially. By inhibiting AdoMetDC, in addition to ODC, 5,8-dimethylspermine markedly reduced the total polyamine content of the cells. As a result, the rate of cell proliferation decreased during the course of treatment. Inhibition of ODC and AdoMetDC in conjunction with the inability of 5,8-dimethylspermine to replace the polyamines in their growth-stimulatory capacity, makes this type of compound particularly interesting.

Acknowledgements

This investigation was supported by grants from the Swedish Natural Science (B-BU 4086-115) and Medical (04X-02212) Research Councils, the Medical Faculty (University of Lund), and the John and Augusta Persson, the Lars Hierta, the Magnus Bergvall, the Berta Kamprad and the Carl Tesdorpf Research Foundations.

References

1 Heby, O. and Jänne, J. (1981) in Polyamines in Biology and Medicine (Morris, D.R. and Marton, L.J., eds.), pp. 243–310, Marcel Dekker, New York.
2 Bey, P., Danzin, C. and Jung, M. (1987) in Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies (McCann, P.P., Pegg, A.E. and Sjoerdsma, A., eds.), pp. 1–31, Academic Press, New York.
3 Kay, J.E. and Lindsay, V.J. (1973) Biochem. J. 132, 791–796.
4 Clark, J.L. (1974) Biochemistry 13, 4668–4674.
5 Jänne, J. and Hölttä, E. (1974) Biochem. Biophys. Res. Commun. 61, 449–456.
6 Fong, W.F., Heller, J.S. and Canellakis, E.S. (1976) Biochim. Biophys. Acta 428, 456–465.
7 Heller, J.S., Fong, W.F. and Canellakis, E.S. (1976) Proc. Natl. Acad. Sci. USA 73, 1858–1862.
8 McCann, P.P., Tardif, C., Hornsperger, J.M. and Böhlen, P. (1979) J. Cell. Physiol. 99, 183–190.
9 Seely, J.E. and Pegg, A.E. (1983) Biochem. J. 216, 701–717.
10 Murakami, Y., Fujita, K., Kameji, T. and Hayashi, S. (1985) Biochem. J. 225, 689–697.
11 Persson, L., Oredsson, S.M., Anehus, S. and Heby, O. (1985) Biochem. Biophys. Res. Commun. 131, 239–245.
12 Kahana, C. and Nathans, D. (1985) J. Biol. Chem. 260, 15390–15393.
13 Persson, L., Holm, I. and Heby, O. (1986) FEBS Lett. 205, 175–178.
14 Hölttä, E. and Pohjanpelto, P. (1986) J. Biol. Chem. 261, 9502–9508.
15 Persson, L., Holm, I. and Heby, O. (1988) J. Biol. Chem. 263, 3528–3533.
16 Seiler, N. (1981) in Polyamines in Biology and Medicine (Morris, D.R. and Marton, L.J., eds.), pp. 127–149, Marcel Dekker, New York.
17 Seiler, N., Bolkenius, F.N. and Rennert, O.M. (1981) Med. Biol. 59, 334–346.
18 Pegg, A.E., Hibasami, H., Matsui, I. and Bethell, D.R. (1981) Adv. Enzyme Reg. 19, 427–451.
19 Sarhan, S., Dezeure, F. and Seiler, N. (1987) Int. J. Biochem. 19, 1037–1047.
20 Metcalf, B.W., Bey, P., Danzin, C., Jung, M.J., Casara, P. and Vevert, J.P. (1978) J. Am. Chem. Soc. 100, 2551–2553.
21 Tabor, H., Tabor, C.W. and De Meis, L. (1971) Methods Enzymol. 17B, 829–833.
22 Jänne, J. and Williams-Ashman, H.G. (1971) Biochem. Biophys. Res. Commun. 42, 222–229.
23 Pösö, H. and Pegg, A.E. (1982) Biochemistry 21, 3116–3122.
24 Persson, L. (1982) Acta Chem. Scand. B36, 685–688.
25 Persson, L., Seely, J.E. and Pegg, A.E. (1984) Biochemistry 23, 3777–3783.
26 Seiler, N. and Knödgen, B. (1985) J. Chromatog. 339, 45–57.
27 Persson, L. and Pegg, A.E. (1984) J. Biol. Chem. 259, 12364–12367.
28 Mamont, P.S., Duchesne, M.-C., Grove, J. and Bey, P. (1978) Biochem. Biophys. Res. Commun. 81, 58–66.
29 Oredsson, S., Anehus, S. and Heby, O. (1980) Biochem. Biophys. Res. Commun. 94, 151–158.

30 Oredsson, S.M., Anehus, S. and Heby, O. (1984) Mol. Cell. Biochem. 64, 163–172.
31 Pegg, A.E. (1986) Biochem. J. 234, 249–262.
32 Seiler, N. and Heby, O. (1988) Acta Biochim. Biophys. Hung. 23, 1–36.
33 Mamont, P.S., Joder-Ohlenbusch, A.-M., Nussli, M. and Grove, J. (1981) Biochem. J. 196, 411–422.
34 Pegg, A.E., Wechter, R. and Pajunen, A. (1987) Biochem. J. 244, 49–54.
35 Shirahata, A. and Pegg, A.E. (1986) J. Biol. Chem. 261, 13833–13837.
36 Kameji, T. and Pegg, A.E. (1987) J. Biol. Chem. 262, 2427–2430.
37 Porter, C.W., McManis, J., Casero, R.A. and Bergeron, R.J. (1987) Cancer Res. 47, 2821–2825.
38 Porter, C.W., Berger, F.G., Pegg, A.E., Ganis, B. and Bergeron, R.J. (1987) Biochem. J. 242, 433–440.