Increase in *S*-adenosylmethionine decarboxylase in SV-3T3 cells treated with *S*-methyl-5'-methylthioadenosine

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Treatment of SV-3T3 cells with the spermine synthase inhibitor *S*-methyl-5'-methylthioadenosine [AdoS⁺(CH₃)₂] led to a large increase in the activity of *S*-adenosylmethionine decarboxylase (AdoMetDC) without affecting ornithine decarboxylase. The elevation of AdoMetDC activity was due to an increased amount of enzyme protein, as demonstrated by radioimmunoassay and by immunoblotting. The increase in AdoMetDC protein was caused by at least three factors: an increase in the amount of translatable mRNA, an increased translation efficiency of the mRNA and an increase in the half-life of the protein. The depletion of spermine brought about by AdoS⁺(CH₃)₂ appeared to be responsible for the increased synthesis of AdoMetDC and for part of the decrease in its rate of degradation. An additional stabilization of the enzyme protein was probably due to the binding of AdoS⁺(CH₃)₂, which is also a weak inhibitor of AdoMetDC. These results demonstrate the importance of cellular spermine concentrations in regulating the activity of AdoMetDC, which is a key enzyme controlling polyamine synthesis.

INTRODUCTION

S-Adenosylmethionine decarboxylase (AdoMetDC) is a key regulatory enzyme in the biosynthesis of the polyamines spermidine and spermine [1–4]. Its activity can be altered by drugs which affect polyamine biosynthesis. Many studies have shown that treatment with MGBG, a potent but reversible inhibitor of AdoMetDC, leads to a large increase in the amount of this enzyme (reviewed in [1–3]). This increase appears to be mediated by a decrease in the rate of degradation of the enzyme protein, which is brought about by the binding of the inhibitor. More recently, it has been discovered that treatment with inhibitors of ODC, such as DFMO [5–7], and of spermidine synthase, such as AdoDato [8], also lead to the accumulation of AdoMetDC. These findings show that the activity of AdoMetDC is tightly regulated by the cellular polyamine content. In accordance with this hypothesis, it has been demonstrated that the administration of polyamines (either spermidine or spermine) decreases AdoMetDC activity [5–9]. The mechanism by which spermidine exerted its effects on the content of this enzyme was studied by using DFMO, and it was found that spermidine concentrations affected both the half-life of the enzyme [5,6,10] and the content of its mRNA [11]. However, it is not possible to evaluate the relative effects of each polyamine separately by the use of AdoDato or DFMO, since spermine content is increased by AdoDato [8] and is largely unaffected by DFMO [4–7]. It has been found that AdoS⁺(CH₃)₂ is a potent inhibitor of spermine synthase and can be used to deplete cells selectively of spermine [12]. We have therefore investigated the effect of AdoS⁺(CH₃)₂ on AdoMetDC in SV-3T3 cells. It was found that exposure to AdoS⁺(CH₃)₂

produced a much larger increase in AdoMetDC than did exposure to DFMO, but that only a small part of this effect was due to an increased content of mRNA.

MATERIALS AND METHODS

Assay of AdoMetDC activity and protein

AdoMetDC was assayed by measuring the release of ¹⁴CO₂ from [*carboxy*-¹⁴C]AdoMet as previously described [7,9]. One unit of activity was defined as the amount needed to release 1 nmol of CO₂ per 30 min, and results were expressed as units/mg of protein. Protein was determined by the method of Bradford [13]. The amount of AdoMetDC protein was determined by radioimmunoassay as described by Shirahata *et al.* [9], by using AdoMetDC labelled by reaction with sodium cyanoborohydride and decarboxylated Ado[³⁵S]Met as the radioactive antigen.

Immunoblotting

Samples of the cell extracts used for assay of AdoMetDC protein were precipitated by the addition of trichloroacetic acid (final concn. 6.8%, w/v) and sodium deoxycholate (final concn. 0.02%, w/v). The pellets were washed twice with ice-cold acetone and dissolved in 62.5 mM-Tris/HCl (pH 6.8)/5% (w/v) SDS/5% (v/v) 2-mercaptoethanol/10% (v/v) glycerol by heating in a boiling-water bath for 5 min. After separation by polyacrylamide-gel electrophoresis [14], the proteins were transferred electrophoretically to nitrocellulose paper (Schleicher & Schuell, BA 85) in a Bio-Rad Trans-Blot cell [15]. Immunoblot detection was carried out with rabbit antiserum against purified AdoMetDC [9] by the method of De Blas & Cherwinski [16].

* Abbreviations used: AdoMet, *S*-adenosylmethionine; AdoMetDC, *S*-adenosylmethionine decarboxylase (EC 4.1.1.50); AdoS⁺(CH₃)₂, *S*-methyl-5'-methylthioadenosine [dimethyl-(5'-adenosyl)sulphonium perchlorate salt]; MTA, 5'-methylthioadenosine; DFMO, DL- α -difluoromethyl-ornithine; ODC, ornithine decarboxylase (EC 4.1.1.17); AdoDato, *S*-adenosyl-1,8-diamino-3-thio-octane; MGBG, methylglyoxal bis(guanyldiazene).

Determination of mRNA content of AdoMetDC

Polyadenylated mRNA was isolated from the cells and translated in a reticulocyte lysate as previously described [11]. The translation products were precipitated with a monospecific antiserum to AdoMetDC [9], separated by polyacrylamide-gel electrophoresis [14] and subjected to fluorography. The developed films were scanned with a scanning densitometer in order to compare the relative intensities of the bands corresponding to AdoMetDC.

Cell culture

SV-3T3 cells were grown in Dulbecco's modified Eagle's medium with 3% horse serum/2% fetal-calf serum as described by Pegg [7]. The experiments were done with concentrations of AdoS⁺(CH₃)₂ that did not greatly inhibit cell growth over a 72 h period, with no effect at up to 0.4 mM, 91% of the control cell number at 0.7 mM and 76% at 1 mM. However, MTA had a significant anti-proliferative effect, such that after 48 h in the MTA-treated cultures there was only 40% of the control cell number at 0.7 mM, 69% at 0.4 mM and 85% at 0.2 mM.

Polyamine analysis

The cells were then harvested and extracted with 10% (w/v) trichloroacetic acid, and samples were used for the determination of polyamine content in an amino acid analyser with fluorescence detection [7,8]. Results are expressed as nmol of polyamine/10⁶ cells. The cell number was determined as previously described [7].

Determination of the intracellular content of decarboxylated AdoMet

Cell extracts were prepared as for polyamine analysis, and the content of AdoMet and its decarboxylated derivative were determined by using an ion-pair reversed-phase separation [17]. Samples (up to 0.25 ml) were mixed with 0.45 ml of buffer A (0.1 M-sodium acetate/0.01 M-sodium octanesulphonate, pH 4.5), centrifuged at 5000 *g* for 10 min, and applied to a column (Beckman Ultrasphere ODS 5 μ m; 4.6 mm $\times$ 25 cm, protected by a 4.6 mm $\times$ 4 cm guard column of ODS-5S from Bio-Rad) equilibrated with a mixture of 90% buffer A and 10% buffer B (10 parts of 0.2 M-sodium acetate/0.01 M-sodium octanesulphonate, pH 4.5; 3 parts of acetonitrile). The column was then eluted with a linear gradient of 90% buffer A/10% buffer B to 100% buffer B over 40 min at a flow rate of 1 ml/min at 35 °C. The eluate was monitored at 254 nm, and the amounts of AdoMet and decarboxylated AdoMet were calculated from the peak heights by using standard curves constructed with known amounts of the authentic compounds. The results were expressed as nmol of nucleoside present/mg of total protein in the cell extracts.

Materials

AdoS⁺(CH₃)₂ was synthesized by methylation of MTA with methyl iodide [18]. Its purity and that of the MTA used were established by h.p.l.c. as described above and were found to be more than 98%. All biochemical reagents were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

RESULTS

When cultured SV-3T3 cells were treated with AdoS⁺(CH₃)₂ for 48 h, there was a striking increase in the activity of AdoMetDC (Table 1). This increase was dose-dependent, with a 7-fold increase occurring with 0.1 mM-AdoS⁺(CH₃)₂ and a 25-fold increase at 1 mM-AdoS⁺(CH₃)₂. MTA also produced an increase in AdoMetDC activity, but was not as active as AdoS⁺(CH₃)₂, giving only a 7-fold increase at 0.7 mM, and because of toxicity could not be tested at higher doses (Table 1). ODC activity was not stimulated by either nucleoside and was actually decreased at the lower doses of AdoS⁺(CH₃)₂ (Table 1). The effect of

Table 1. Effect of AdoS⁺(CH₃)₂ on AdoMetDC activity in SV-3T3 cells

Drugs were added 5 h after plating and activities were measured 48 h after plating. Results are given as means $\pm$ s.d. for at least four estimations.

Addition	Activity (units/mg of protein)	
	AdoMetDC	ODC
None	0.7 $\pm$ 0.1	16.0 $\pm$ 2.7
0.1 mM-AdoS ⁺ (CH ₃) ₂	4.8 $\pm$ 0.6	9.6 $\pm$ 1.1
0.2 mM-AdoS ⁺ (CH ₃) ₂	5.8 $\pm$ 1.6	8.7 $\pm$ 2.2
0.4 mM-AdoS ⁺ (CH ₃) ₂	9.5 $\pm$ 1.1	15.6 $\pm$ 1.5
0.7 mM-AdoS ⁺ (CH ₃) ₂	13.5 $\pm$ 3.2	11.0 $\pm$ 0.6
1.0 mM-AdoS ⁺ (CH ₃) ₂	17.6 $\pm$ 4.6	11.8 $\pm$ 0.8
5 mM-DFMO	4.7 $\pm$ 0.6	< 0.1
0.2 mM-MTA	1.8 $\pm$ 0.4	14.8 $\pm$ 2.2
0.4 mM-MTA	4.0 $\pm$ 0.2	15.8 $\pm$ 2.8
0.7 mM-MTA	5.1 $\pm$ 0.7	9.3 $\pm$ 1.4

Fig. 1. Time course of change in AdoMetDC activity produced by AdoS⁺(CH₃)₂ in SV-3T3 cells

The cells were grown for 24 h. At this time one group received no addition (■) and the other group was treated with 0.4 mM-AdoS⁺(CH₃)₂ (●). Cells were harvested at various times later as shown and the activity of AdoMetDC was measured.

Table 2. Effects of AdoS⁺(CH₃)₂ on polyamines in SV-3T3 cells

The cells were grown for 24 h, and at this time the additions were made. The time given refers to the number of hours after the time of addition at which the cells were harvested and the AdoMetDC activity was determined. The culture medium contained 1 mM-aminoguanidine to prevent oxidation of the added spermine. Results are given $\pm$ S.D. for at least four estimations: ND, not determined.

Treatment	Time (h)	Polyamine (nmol/10 ⁶ cells)			AdoMetDC activity (units/mg)
		Putrescine	Spermidine	Spermine	
None	0	0.61 $\pm$ 0.19	3.20 $\pm$ 0.12	1.18 $\pm$ 0.31	0.7 $\pm$ 0.1
None	16	0.66 $\pm$ 0.07	3.38 $\pm$ 0.35	1.11 $\pm$ 0.11	1.2 $\pm$ 0.3
None	32	0.59 $\pm$ 0.12	2.45 $\pm$ 0.68	0.93 $\pm$ 0.23	0.9 $\pm$ 0.1
0.4 mM-AdoS ⁺ (CH ₃) ₂	16	1.82 $\pm$ 0.39	3.79 $\pm$ 0.19	0.46 $\pm$ 0.04	14.4 $\pm$ 1.4
0.4 mM-AdoS ⁺ (CH ₃) ₂	32	1.03 $\pm$ 0.11	3.34 $\pm$ 0.45	0.17 $\pm$ 0.02	11.5 $\pm$ 1.2
1 μ M-spermine + 0.4 mM-AdoS ⁺ (CH ₃) ₂	16	0.62 $\pm$ 0.25	2.56 $\pm$ 0.16	2.59 $\pm$ 0.24	3.5 $\pm$ 0.3
2.5 μ M-spermine + 0.4 mM-AdoS ⁺ (CH ₃) ₂	16	0.23 $\pm$ 0.10	1.54 $\pm$ 0.28	3.58 $\pm$ 0.52	1.5 $\pm$ 0.2
5 μ M-spermine + 0.4 mM-AdoS ⁺ (CH ₃) ₂	16	0.15 $\pm$ 0.05	1.22 $\pm$ 0.26	3.51 $\pm$ 0.38	1.3 $\pm$ 0.2
10 μ M-spermine + 0.4 mM-AdoS ⁺ (CH ₃) ₂	16	0.16 $\pm$ 0.02	1.08 $\pm$ 0.07	3.81 $\pm$ 0.26	1.0 $\pm$ 0.09
10 μ M-spermine + 0.4 mM-AdoS ⁺ (CH ₃) ₂	32	< 0.05	0.64 $\pm$ 0.13	2.80 $\pm$ 0.10	0.3 $\pm$ 0.08
10 μ M-spermine	16	ND	ND	ND	0.09 $\pm$ 0.02
10 μ M-spermine	32	ND	ND	ND	0.03 $\pm$ 0.01

AdoS⁺(CH₃)₂ on AdoMetDC activity was considerably greater than that of the ODC inhibitor DFMO, which increased AdoMetDC by only 7-fold.

A time course of the induction of AdoMetDC by 0.4 mM-AdoS⁺(CH₃)₂ showed that a maximal increase required about 24 h of exposure and that activity remained at the high value for at least a further 16 h (Fig. 1).

The polyamine content of the SV-3T3 cells in the experiment shown in Fig. 1 is given in Table 2. In agreement with previous reports [11,19], AdoS⁺(CH₃)₂ led to a marked decline in cellular spermine content, which amounted to about an 85% decrease within 32 h. The time of maximal stimulation of AdoMetDC corresponded well to the decrease in spermine content (Fig. 1). Provision of exogenous spermine as well as AdoS⁺(CH₃)₂ completely reversed the decline in spermine and prevented the accumulation of AdoMetDC (Table 2).

The increase in AdoMetDC activity brought about by AdoS⁺(CH₃)₂ was clearly due to an increased amount of the AdoMetDC protein, as measured by radioimmunoassay (Table 3) and immunoblotting (Fig. 2). The constant ratio between AdoMetDC activity and protein seen when extracts from cells treated with various amounts of AdoS⁺(CH₃)₂ were tested by radioimmunoassay indicates that any residual drug present in the cell extracts was not sufficient to exert any inhibitory effects on the assays of activity. The immunoblots of protein taken from these cells and then subjected to polyacrylamide-gel electrophoresis under denaturing conditions showed only a single band, corresponding to an *M_r* of 32000 (Fig. 2), which agrees with our earlier values for the subunit of AdoMetDC from SV-3T3 cells and other mammalian cells [4,9]. There was no indication of any change in the size of the AdoMetDC protein after stimulation by AdoS⁺(CH₃)₂ (Fig. 2).

The amounts of AdoMetDC mRNA were determined by the preparation of polyadenylated RNA and its translation in a reticulocyte lysate system, followed by precipitation of the protein corresponding to AdoMetDC with a specific antiserum. These proteins were then

separated by polyacrylamide-gel electrophoresis. The products detected in this way consisted of two bands, of *M_r* 32000 and 37000 (Fig. 3). These corresponded exactly to the subunit of AdoMetDC and to a precursor which we have previously identified in experiments using mRNA for AdoMetDC from rat prostate [11]. The amount of mRNA for AdoMetDC was therefore determined by densitometric scanning of the two bands. As shown in Fig. 4 and quantified in Table 4, the amount of translatable mRNA for AdoMetDC was increased by exposure to AdoS⁺(CH₃)₂. However, the increase in mRNA content was only 2–3-fold and could not account for all of the 10–30-fold increase in activity. This increase in mRNA was completely abolished by the provision of exogenous spermine.

The effects of drugs which depleted cellular spermidine on the AdoMetDC mRNA content were also examined (Table 4). The increase in AdoMetDC in response to DFMO was accompanied by a 3-fold increase in the amount of translatable mRNA, which is almost enough to account for the 4.5-fold rise in activity which occurred in this experiment. Similarly, AdoDato doubled the content of mRNA and tripled the amount of AdoMetDC.

Table 3. Effect of AdoS⁺(CH₃)₂ on AdoMetDC protein in SV-3T3 cells

Cells were grown for 48 h in the presence of the concentration of AdoS⁺(CH₃)₂ shown. Results are given as means $\pm$ S.D. for at least four estimations.

AdoS ⁺ (CH ₃) ₂ concn. added	AdoMetDC		Ratio (activity/protein)
	Activity (units/mg)	Protein (pmol/mg)	
None	0.63 $\pm$ 0.03	0.19 $\pm$ 0.02	3.3
0.1 mM	4.8 $\pm$ 0.6	1.1 $\pm$ 0.2	4.3
0.2 mM	7.0 $\pm$ 1.2	1.6 $\pm$ 0.3	4.4
0.4 mM	10.8 $\pm$ 1.6	2.6 $\pm$ 0.6	4.1
0.8 mM	15.8 $\pm$ 5.4	4.4 $\pm$ 1.1	3.6

Fig. 2. Effect of $\text{AdoS}^+(\text{CH}_3)_2$ on AdoMetDC protein as demonstrated by immunoblotting

Lanes 1–5 were developed with antiserum to AdoMetDC, and lanes 6 and 7 with a control rabbit antiserum. Lane 1 shows results with a marker of purified AdoMetDC (the band of M_r below 30000 is probably a degradation product, since this protein had been stored for several months); lane 2 contained 264 μg of protein from control SV-3T3 cells; lanes 3 and 6 contained 199 μg of protein from SV-3T3 cells treated with 0.4 mM- $\text{AdoS}^+(\text{CH}_3)_2$ for 48 h; lanes 4 and 7 contained 204 μg of protein with SV-3T3 cells treated with 0.4 mM- $\text{AdoS}^+(\text{CH}_3)_2$ for 48 h; lane 5 had no protein added. The arrows indicate the positions of M_r standards.

Another factor which may account for the very large increase in AdoMetDC in response to $\text{AdoS}^+(\text{CH}_3)_2$ is the possible stabilization of the protein against intracellular degradation. As shown in Fig. 5, the half-life of AdoMetDC was greatly increased in cells treated with $\text{AdoS}^+(\text{CH}_3)_2$. In control cells, the half-life was 66 ± 10 min. This was increased to 153 ± 16 min in cells treated with 0.2 mM- $\text{AdoS}^+(\text{CH}_3)_2$ and to more than 6 h in cells treated with 0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$. The replacement of cellular spermine content only partially reversed this effect. The addition of 1 μM -spermine to cells treated with 0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$ decreased the half-life to 220 ± 20 min (Fig. 5).

These results suggest that the binding of $\text{AdoS}^+(\text{CH}_3)_2$ to AdoMetDC may stabilize the protein against its normal intracellular degradation. If this is the case, it raises the question as to why the addition of 10 μM -spermine completely overcame the rise in AdoMetDC activity, since spermine did not restore the half-life to normal values (Fig. 5) and only decreased the mRNA content by at most a factor of 2 (Table 3). A possible explanation is shown by the results in lanes 6 and 7 of Fig. 4, in which spermine was added to the reticulocyte system and its effect on the translation of AdoMetDC from cells grown in the presence of 0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$ was determined. In the presence of 0.1 mM-spermine the

Fig. 3. Translation of SV-3T3-cell mRNA for AdoMetDC in reticulocyte lysates

For each sample from SV-3T3 cells 5 μg of polyadenylated RNA was translated in a reticulocyte lysate. The samples were then treated with antiserum to AdoMetDC (lanes 1–3) or control antiserum (lanes 4 and 5), separated by polyacrylamide-gel electrophoresis and subjected to fluorography. Lanes 1 and 4 show results for control SV-3T3 cells (2×10^6 c.p.m., subjected to immunoprecipitation); lanes 2 and 5 show results for SV-3T3 cells treated with 0.4 mM- $\text{AdoS}^+(\text{CH}_3)_2$ for 48 h (1.8×10^6 c.p.m.); lane 3 shows results for a preparation of 1 μg of mRNA [11] from rat prostate (2.1×10^6 c.p.m.). The arrows indicate the positions of M_r standards.

synthesis of AdoMetDC was much decreased, even though total protein synthesis was not greatly affected. These results indicate that the synthesis of AdoMetDC is regulated at the translational level by polyamines.

DISCUSSION

Our results indicate that the increase in AdoMetDC activity in response to $\text{AdoS}^+(\text{CH}_3)_2$ is brought about by an increased amount of AdoMetDC protein. The results of the radioimmunoassays and the immunoblots indicate no change in the protein indicative of post-translational modifications leading to a change in the catalytic activity. The rise in AdoMetDC protein appears to be mediated by at least three factors. These are: (a) a stabilization of the protein, leading to an increase in its half-life; (b) an increase in the content of mRNA for AdoMetDC; and (c) an increased translational efficiency of the AdoMetDC mRNA.

Fig. 4. Effect of treatment with $\text{AdoS}^+(\text{CH}_3)_2$ and of addition of spermine on translation of SV-3T3-cell mRNA for AdoMetDC in reticulocyte lysates

After translation of 5 μg of polyadenylated RNA in a reticulocyte lysate, the samples were treated with antiserum to AdoMetDC, separated by polyacrylamide-gel electrophoresis and subjected to fluorography. Lane 1 shows results for control SV-3T3 cells (1.5×10^6 c.p.m., subjected to immunoprecipitation); lanes 2 and 3 show results for SV-3T3 cells treated with 0.4 mM- $\text{AdoS}^+(\text{CH}_3)_2$ for 48 h (1.6×10^6 c.p.m. and 1.5×10^6 c.p.m. respectively); lane 4 shows results for SV-3T3 cells treated with 0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$ for 48 h (2.0×10^6 c.p.m.); lane 5 shows a marker of authentic ^{35}S -AdoMetDC; lane 6 shows results for SV-3T3 cells treated with 0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$ for 48 h (2.0×10^6 c.p.m.); and lane 7 shows results for the same mRNA as in lane 6, but the translation was carried out in the presence of 0.1 mM-spermine (1.96×10^6 c.p.m.). It should be noted that the experiments in lanes 1–4 and 5–7 were not carried out at the same time, and the extent of film development may not be the same. The arrows indicate the positions of M_r standards.

Experiments in which spermine was administered to replenish the cellular spermine pools depleted by $\text{AdoS}^+(\text{CH}_3)_2$ indicate that the stabilization of the protein appears to be mediated both by the binding of $\text{AdoS}^+(\text{CH}_3)_2$ (which is a weak inhibitor of the enzyme [19,20]) and by the decline in cellular spermine. The additional increase in AdoMetDC which occurs in response to high concentrations (above 0.4 mM) of $\text{AdoS}^+(\text{CH}_3)_2$ may be due solely to this effect, since these concentrations have little additional effect on cellular spermine [12]. In contrast, the increases in AdoMetDC mRNA and in its translation efficiency appear to be a response solely to spermine depletion.

Although MTA itself is known to be a potent inhibitor of spermine synthase *in vitro* [4,21], $\text{AdoS}^+(\text{CH}_3)_2$ is much more active in depleting cellular spermine in SV-3T3 cells [12], because MTA is very rapidly degraded by the action of MTA phosphorylase [4,22,23]. The results shown in Table 1 confirm that high concentrations of MTA also lead to an increase in AdoMetDC activity, but even at 0.7 mM-MTA the effect was less than that of 0.2 mM- $\text{AdoS}^+(\text{CH}_3)_2$. We were not able to test higher

Table 4. Effect of $\text{AdoS}^+(\text{CH}_3)_2$ and other inhibitors on AdoMetDC activity and mRNA in SV-3T3 cells

Cells were grown as in Table 3. Results are shown as means of two observations or as means $\pm$ S.D. for three or more estimations.

Treatment	AdoMetDC activity (units/mg)	AdoMetDC mRNA (relative units)*
None	0.5 ± 0.1	1.0
0.1 mM- $\text{AdoS}^+(\text{CH}_3)_2$	5.0 ± 0.4	1.5 ± 0.2
0.4 mM- $\text{AdoS}^+(\text{CH}_3)_2$	10.9 ± 2.0	2.0 ± 0.4
0.4 mM- $\text{AdoS}^+(\text{CH}_3)_2$ + 10 μM -spermine	0.48	0.5
0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$	15.0 ± 2.4	2.8 ± 0.7
0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$ + 1 μM -spermine	7.4	0.4
5 mM-DFMO	2.2	3.0 ± 0.8
0.1 mM-AdoDato	1.7	2.1
0.1 mM-AdoDato + 10 μM spermidine	0.3	1.0

* The densitometer reading for the control cell values was set at a value of 1.

Fig. 5. Effect of $\text{AdoS}^+(\text{CH}_3)_2$ on turnover of AdoMetDC mRNA

The half-life of AdoMetDC was determined by the addition of 0.18 mM-cycloheximide to block protein synthesis, and the enzyme activity was measured at various times later as shown. Results are plotted for control cells (●), cells treated with 0.2 mM- $\text{AdoS}^+(\text{CH}_3)_2$ (+0.2 mM, ▲), cells treated with 0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$ (+0.7 mM, ■) and cells treated with 0.7 mM- $\text{AdoS}^+(\text{CH}_3)_2$ plus 1 μM -spermine (+0.7 mM + SPM, ◆).

concentrations of MTA, since these exerted significant toxic effects on the SV-3T3 cells which could not be overcome by the addition of polyamines, indicating that MTA at these concentrations influences other cellular metabolic processes (R. Wechter & A. E. Pegg, unpublished work). However, Carson and his collaborators have found that MTA led to a rapid rise in AdoMetDC activity when added to mouse lymphoma cells deficient in MTA phosphorylase and resistant to MTA toxicity [23].

Comparison of the effect of AdoS⁺(CH₃)₂, which depletes spermine, with the effects of DFMO and AdoDato, which deplete spermidine, suggest that spermidine is the more potent polyamine in regulating the content of mRNA for AdoMetDC (Table 4; [8,11,12]). A more detailed study of the effects of polyamines on the synthesis of ODC and AdoMetDC in reticulocyte lysates freed from endogenous polyamines by gel filtration indicated that spermine appeared to be more effective as an inhibitor of the translation of AdoMetDC mRNA than was spermidine [24].

The multifaceted nature of the regulation of AdoMetDC by the polyamines spermidine and spermine is consistent with the key role of this enzyme in the maintenance of cellular polyamine concentrations. A decrease in spermidine is likely to lead to a sustained increase in the amount of this enzyme by virtue of an increase in the mRNA. A decrease in spermine could produce a more rapid response by increasing the efficiency of translation of the existing mRNA. Both polyamines act to increase the rate of degradation of the protein, providing a rapid method for removing excess enzyme when polyamine content exceeds the physiological range.

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REFERENCES

1. Tabor, C. W. & Tabor, H. (1984) *Adv. Enzymol. Relat. Areas Mol. Biol.* **56**, 251–282
2. Pegg, A. E. (1984) *Cell Biochem. Func.* **2**, 11–15
3. Williams-Ashman, H. G. & Pegg, A. E. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 407–436, Marcel Dekker, New York
4. Pegg, A. E. (1986) *Biochem. J.* **234**, 249–262
5. Alhonen-Hongisto, L. (1980) *Biochem. J.* **190**, 747–754
6. Mamont, P. S., Joder-Ohlenbusch, A.-M., Nussli, M. & Grove, J. (1981) *Biochem. J.* **196**, 411–422
7. Pegg, A. E. (1984) *Biochem. J.* **224**, 29–38
8. Pegg, A. E., Tang, K.-C. & Coward, J. K. (1982) *Biochemistry* **21**, 5082–5089
9. Shirahata, A., Christman, K. & Pegg, A. E. (1985) *Biochemistry* **24**, 4417–4423
10. Shirahata, A. & Pegg, A. E. (1985) *J. Biol. Chem.* **260**, 9583–9588
11. Shirahata, A. & Pegg, A. E. (1986) *J. Biol. Chem.* **261**, 13833–13837
12. Pegg, A. E. & Coward, J. K. (1985) *Biochem. Biophys. Res. Commun.* **133**, 82–89
13. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
14. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
15. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
16. De Blas, A. L. & Cherwinski, H. M. (1983) *Anal. Biochem.* **133**, 214–219
17. Seiler, N. (1983) *Methods Enzymol.* **94**, 25–29
18. Tang, K.-C., Pegg, A. E. & Coward, J. K. (1980) *Biochem. Biophys. Res. Commun.* **96**, 1371–1377
19. Pegg, A. E., Coward, J. K., Talekar, R. R. & Secrist, J. A. (1986) *Biochemistry* **25**, 4091–4097
20. Kolb, M., Danzin, C., Barth, J. & Claverie, N. (1982) *J. Med. Chem.* **25**, 550–556
21. Pajula, R.-L. (1983) *Biochem. J.* **215**, 669–676
22. Pegg, A. E. & McCann, P. P. (1982) *Am. J. Physiol.* **243**, C212–C221
23. Kubota, M., Kajander, E. O. & Carson, D. A. (1985) *Cancer Res.* **45**, 3567–3572
24. Kameji, T. & Pegg, A. E. (1987) *J. Biol. Chem.*, in the press

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