Biochem. J. (1986) 238, 581–587 (Printed in Great Britain)

Regulation of spermidine/spermine N^1-acetyltransferase in L6 cells by polyamines and related compounds

Bradley G. ERWIN and Anthony E. PEGG
Department of Physiology and Cancer Research Center, Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, P.O. Box 850, Hershey, PA 17033, U.S.A.

Exposure of rat L6 cells in culture to exogenous polyamines led to a very large increase in the activity of spermidine/spermine N^1-acetyltransferase. Spermine was more potent than spermidine in bringing about this increase, but in both cases the elevated acetyltransferase activity increased the cellular conversion of spermidine into putrescine. The N^1-acetyltransferase turned over very rapidly in the L6 cells, with a half-life of 9 min after spermidine and 18 min after spermine. A wide variety of synthetic polyamine analogues also brought about a substantial induction of spermidine/spermine N^1-acetyltransferase activity. These included *sym*-norspermidine, *sym*-norspermine, *sym*-homospermidine, N^4-substituted spermidine derivatives, 1,3,6-triaminohexane, 1,4,7-triaminoheptane and deoxyspergualin, which were comparable with spermidine in their potency, and N^1N^8-bis(ethyl)spermidine, N^1N^9-bis(ethyl)homospermidine, methylglyoxal bis(guanylhydrazone), ethylglyoxal bis(guanylhydrazone) and 1,1'-[(methylethanediylidene)dinitrilo]bis(3-aminoguanidine), which were even more active than spermidine. It is suggested that these polyamine analogues may bring about a decrease in cellular polyamines not only by inhibiting biosynthesis but by stimulating the degradation of spermidine into putrescine.

INTRODUCTION

Previous work in our laboratory has shown that rat liver contains a cytosolic enzyme termed spermidine/spermine N^1-acetyltransferase (SAT) (Matsui *et al.*, 1981; Della Ragione & Pegg, 1982, 1983; Persson & Pegg, 1984). This enzyme uses acetyl-CoA as a substrate and can act on either spermidine or spermine to form the N^1-acetyl derivative. SAT has also been detected in other rat tissues such as kidney, lung, brain and pancreas (Matsui & Pegg, 1982; Danzin *et al.*, 1982; Persson & Pegg, 1984), in chicken duodenum (Shinki *et al.*, 1985), in bovine lymphocytes (Matsui-Yuasa *et al.*, 1984) and in BHK cells (Wallace *et al.*, 1985). The function of SAT is unclear, but the acetylated spermidine or spermine derivatives formed by SAT are very good substrates for a FAD-dependent polyamine oxidase which splits off the acetylated aminopropyl group as acetylamidopropanal, thus converting N^1-acetylspermidine into putrescine and N^1-acetylspermine into spermidine (Hölttä, 1977; Bolkenius & Seiler, 1981; Seiler *et al.*, 1981*a*,*b*). The combined actions of SAT and polyamine oxidase therefore bring about the conversion of the polyamines into their biosynthetic precursor putrescine. SAT activity appears to be the limiting factor in this conversion. The basal activity of SAT in unstimulated rat tissues is very low, but it is very highly inducible in rat liver in response to CCl_4 and other hepatotoxins (Matsui *et al.*, 1981; Della Ragione & Pegg, 1984; Persson & Pegg, 1984). Such treatments cause the degradation of cellular spermidine into putrescine by the acetylase/oxidase pathway, but the physiological significance of this degradation is not known.

It has been shown that administration of the polyamines, spermidine or spermine to rats leads to a large induction of SAT in liver, kidney, lung and some other tissues (Persson & Pegg, 1984; Pegg & Erwin, 1985). These results suggested that the acetylase/oxidase pathway may be used to regulate the cellular polyamine content by increasing in activity in response to an excess of intracellular polyamines and thereby causing their degradation to putrescine.

It was reported by Stoschek *et al.* (1982) that addition of spermidine to rat L6 myoblast cells in culture produced an increase in cellular putrescine content. In the present paper we describe experiments designed to investigate the inducibility of SAT in these cells and the role of this induction in the degradation of the polyamines into putrescine.

MATERIALS AND METHODS

Materials

Tissue-culture dishes were purchased from Corning Glass Works, Corning, NY, U.S.A. Tissue-culture medium and serum were purchased from Flow Laboratories, McLean, VA, U.S.A. Chick-embryo extract was purchased from GIBCO Laboratories, Grand Island, NY, U.S.A. (Some batches of this extract contained considerable amounts of spermidine and spermine and were dialysed extensively to remove these polyamines before use.) [1-^{14}C]Acetyl-CoA (sp. radioactivity 55 Ci/mol) was purchased from Amersham Corp., Arlington Heights, IL, U.S.A. Liquid-scintillation cocktail (Econofluor) was purchased from New England Nuclear, Boston, MA, U.S.A. DFMO was generously

Abbreviations used: SAT, spermidine/spermine N^1-acetyltransferase; DFMO, DL-2-difluoromethylornithine; MGBG, methylglyoxal bis(guanylhydrazone); EGBG, ethylglyoxal bis(guanylhydrazone); MBAG, 1,1'-[(methylethanediylidene)dinitrilo]bis(3-aminoguanidine); CNSA, *N*-[2-(*S*-CoA)acetyl]*sym*-norspermidine amide.

given by Dr. P. P. McCann, Merrell Dow Research Center, Cincinnati, OH, U.S.A. Putrescine, spermidine, spermine, N^1-acetylspermidine and N^1-acetylspermine were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. *sym*-Norspermidine and *sym*-norspermine were purchased from Eastman Kodak Co., Rochester, NY, U.S.A. MGBG and 1,3-diaminopropane were purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. CNSA was synthesized as previously described (Erwin *et al.*, 1984). Deoxyspergualin was obtained from Dr. J. Plowman, National Cancer Institute, Bethesda, MD, U.S.A. 1,3,6-Triaminohexane and 1,4,7-triaminoheptane were kindly provided by Dr. N. Seiler, Merrell Dow Research Institute, Strasbourg, France. N^1N^8-Bis(ethyl)spermidine, N^1N^9-bis(ethyl)homospermidine, N^4-methylspermidine, *sym*-homospermidine and N^4-acetylspermidine were synthesized by Dr. R. Bergeron, University of Florida, Gainesville, FL, U.S.A. EGBG was provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD, U.S.A. MBAG was synthesized by a published method (Pegg, 1983).

Cell culture and preparation of cell extracts

Stock cultures of L6 myoblasts (Yaffe, 1968) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) horse serum, 1% chick-embryo extract, 30 mM-$NaHCO_3$, penicillin (9 units/ml), and streptomycin (9 μg/ml) in a humidified atmosphere of air/CO_2 (9:1) at 37 °C. Experimental cultures were seeded at a density of 1.13×10^4 cells/cm^2 in medium containing 1 mM-DFMO; 3 days later, after limited cell proliferation had occurred, additions were made to the medium. The time of these additions was designated zero time, and the cells were harvested at various times later which are described in the legends. Harvests were carried out as follows. The medium was removed and the cell surface was washed extensively with ice-cold phosphate-buffered saline (120 mM-NaCl, 12 mM-Na_2HPO_4, 1.5 mM-KH_2PO_4, pH 7.4). The washes and all subsequent extraction procedures were performed at 4 °C. Extracts for enzyme assay were prepared by lysing the cells in a hypo-osmotic buffer consisting of 50 mM-Tris (pH 7.5), 2.5 mM-dithiothreitol and 0.1 mM-EDTA. The resulting extract was frozen in liquid N_2 and rapidly thawed. This was followed by centrifugation at 17000 *g* for 20 min. The supernatant was removed and stored at −20 °C before enzyme assay. Extracts for polyamine analysis were prepared by lysing the cells in 10% (w/v) trichloroacetic acid. Protein precipitation was allowed to occur for at least 4 h, after which the suspension was centrifuged at 15600 *g* for 5 min. The supernatant was stored at −20 °C before polyamine analysis, and the pellet was retained for protein determination.

Assay of spermidine acetyltransferase activity

Total spermidine acetyltransferase activity (referred to as acetyltransferase activity) was assayed in a volume of 100 μl containing 100–400 μg of extract protein, 300 nmol of spermidine, 0.8 nmol of [1-^{14}C]acetyl-CoA, 5 μmol of Tris (pH 7.8) as previously described (Erwin *et al.*, 1984). A unit of activity is defined as 1 pmol of product formed/10 min. Acetyltransferase activity attributable specifically to spermidine/spermine N^1-acetyltransferase (referred to as SAT) was determined by subjecting 100–400 μg of extract protein to either a control rabbit serum or a monospecific antiserum against SAT (Persson & Pegg, 1984). The immunoprecipitation procedure has been described previously (Erwin *et al.*, 1984). SAT activity was calculated for each sample by subtracting the residual activity after immunoprecipitation with monospecific antiserum to SAT from the activity obtained after exposure to a control rabbit serum.

Table 1. Properties of L6-cell acetyltransferase induced by spermidine

L6 cells were grown for 3 days in the presence of 1 mM-DFMO and exposed to 100 μM-spermidine for 9 h as indicated. The extracts were then assayed for acetyltransferase activity in the presence of the concentration of CNSA shown or after precipitation with antiserum to SAT as indicated.

Treatment	Addition	Acetyltransferase activity (units/mg)
None	None	3.0 (100%)
	5 μM-CNSA	0.5 (17%)
	0.5 μM-CNSA	2.3 (77%)
	Antiserum to SAT	2.9 (96%)
Spermidine	None	59 (100%)
	5 μM-CNSA	3.6 (6%)
	0.5 μM-CNSA	23 (40%)
	Antiserum to SAT	8.8 (15%)

Additional analytical procedures

Polyamines were determined with a Dionex (Sunnyvale, CA, U.S.A.) D-500 amino acid analyser by the method of Seidenfeld & Marton (1979). Protein was measured by the method of Bradford (1976), with bovine serum albumin as a standard. The enzyme activities were expressed as units per mg of soluble protein added, and polyamine concentrations were expressed as a function of the total cellular protein.

RESULTS

Extracts were prepared from rat L6 myoblasts and assayed for the ability to catalyse the production of acetylated spermidine from [1-^{14}C]acetyl-CoA and spermidine. As shown in Table 1, there was a substantial increase in this activity when spermidine was added to the cell cultures 9 h before harvest. The increased acetylation was due to an increase in SAT, because (*a*) the induced activity was almost completely precipitated by treatment with a specific antiserum to SAT and (*b*) it was highly sensitive to inhibition by CNSA, which is a potent inhibitor of SAT (Table 1). In contrast, the basal activity in the untreated cells was mainly due to other enzymes, because it was less sensitive to inhibition by CNSA and was not lost on treatment with the antiserum to SAT (Table 1).

The time course of the induction of SAT by spermidine is shown in Fig. 1. A significant increase was seen within 2 h of treatment and a maximal response was produced by 8 h. This peak value represents a more than 70-fold increase in immunoprecipitable SAT. Spermine was even more active as an inducer of SAT, giving a time

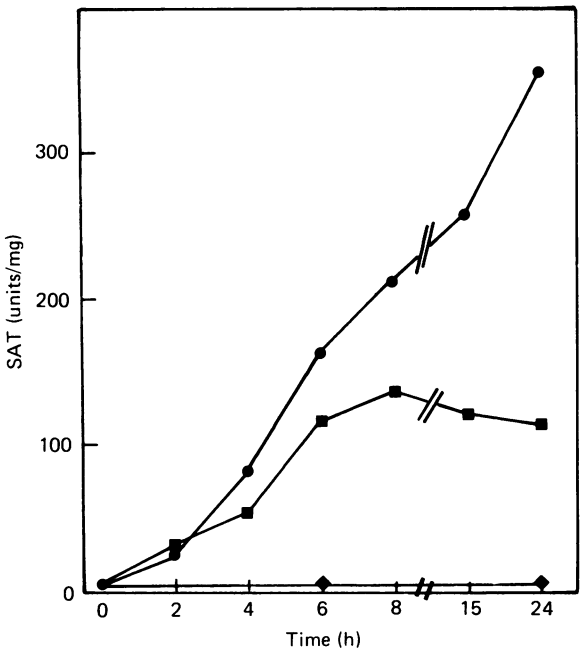

Fig. 1. Time course of induction of SAT by spermidine or spermine

L6 cells were grown in the presence of DFMO for 3 days as described in Table 1, and 100 μM-spermidine (●) or 100 μM-spermine (■) was added, or no addition was made (◆), and the cells were harvested at the time shown. The amount of immunoprecipitable SAT activity in the cell extracts was then determined.

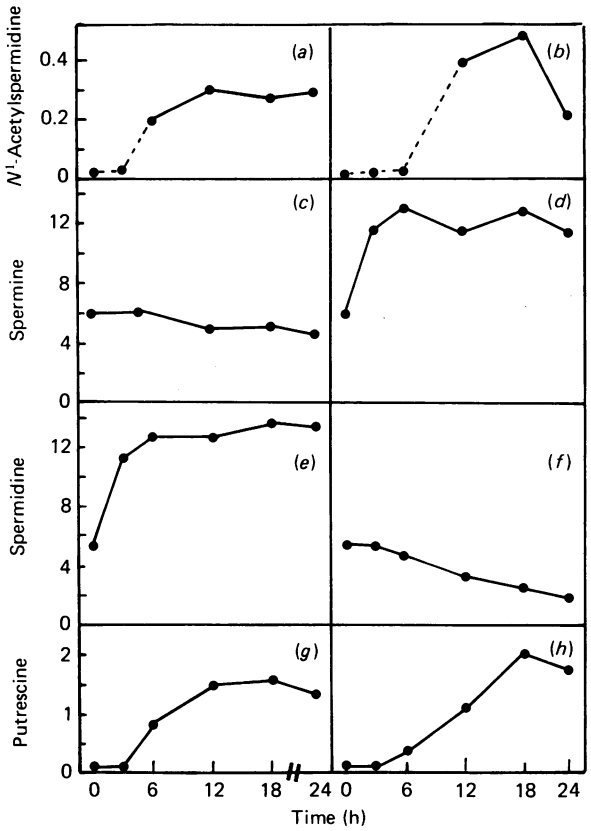

Fig. 2. Effect of spermidine and spermine on polyamine content of L6 cells

The cells were treated as in Fig. 1, and at the times shown the contents of N^1-acetylspermidine (*a* and *b*), spermine (*c* and *d*), spermidine (*e* and *f*) and putrescine (*g* and *h*) were determined and expressed as nmol/mg of protein in the cells. N^1-Acetylspermine was below the limit of detection (about 0.1 nmol/mg) at all times, and the N^1-acetylspermidine points shown as below 0.1 nmol/mg were also below the limit of detection. Results in panels (*a*), (*c*), (*e*) and (*g*) are for cells treated with 100 μM-spermidine, and results in panels (*b*), (*d*), (*f*) and (*h*) are for cells treated with 100 μM-spermine.

course which increased over the entire 24 h period, and there was a more than 180-fold increase in SAT at 24 h after treatment with 100 μM-spermine (Fig. 1). It was not possible to determine the activity reliably at longer time points, as the prolonged exposure to spermine led to some toxic effects, including detachment of the cells from the dishes. The dose–response curve for SAT induction after 9 h exposure indicated that spermidine exerted its greatest effects on SAT when added at concentrations of 30–100 μM. Spermine led to a greater induction at all concentrations, but a maximal effect was obtained with 100 μM (results not shown).

The effect of treatment with 100 μM-spermidine or spermine on cellular polyamine contents is shown in Fig. 2. The protocol of exposure to DFMO before treatment decreased putrescine to 3% of that in control cells and spermidine to 29% of control, and had no significant effect on spermine. Exposure to spermidine led to a substantial increase in both N^1-acetylspermidine and in putrescine at all times after 3 h of exposure (Figs. 2*a* and 2*g*). These results are consistent with the action of the induced SAT on cellular spermidine and subsequent degradation of the N^1-acetylspermidine to putrescine by polyamine oxidase. Treatment with spermine also brought about an increase in putrescine and N^1-acetylspermidine (Figs. 2*b* and 2*h*), but the rise occurred at a later time than with spermidine (cf. Figs. 2*b* and 2*h* with Figs. 2*a* and 2*g*). This correlates with the slower induction of the SAT by spermine. The exposure to spermine also produced a substantial decrease of spermidine in the cells over the time period during which putrescine and N^1-acetylspermidine were increasing.

A variety of polyamines and analogues were tested for the ability to induce SAT in the L6 cells (Table 2). The diamines, putrescine and 1,3-diaminopropane, were virtually inactive, but almost all of the other compounds tested were found to be inducers. These compounds included 1,3,6-triaminohexane (which was slightly less active than spermidine) and 1,4,7-triaminoheptane (which was more active than either spermidine or spermine), showing that a secondary amino group is not essential for induction.

sym-Norspermine, *sym*-norspermidine and *sym*-homospermidine were slightly less active than spermidine itself. Additions of a benzyl or acetyl substitutent to N-4 of spermidine decreased the activity, although N^4-methylspermidine was about as active as spermidine itself. However, additions to the terminal amino groups increased the inducing activity, since N^1N^8-bis(ethyl)-spermidine and N^1N^9-bis(ethyl)homospermidine were considerably more active than spermidine or *sym*-homospermidine (Table 2).

Table 2. Effect of various polyamines and analogues in the induction of SAT

L6 cells were grown in the presence of DFMO for 3 days and then exposed to the concentration of the amine shown for 6 h (Expt. A) or for 9 h (Expt. B). Extracts were then prepared and assayed for total acetyltransferase activity (centre column) or for the acetyltransferase which is immunoprecipitable by the antiserum to SAT (right-hand column).

	Inducer	Acetyltransferase activity (units/mg)	SAT activity (units/mg)
A (measured at 6 h)	None	5	0.4
	100 μM-spermidine	37±3	33±3
	100 μM-spermine	67±23	60±21
	100 μM-*sym*-norspermidine	31±5	26±4
	100 μM-*sym*-norspermine	33±7	30±6
	100 μM-*sym*-homospermidine	24±1	23±1
	100 μM-putrescine	7.8	7.4
	100 μM-1,3-diaminopropane	7.1	6.7
	5 μM-MGBG	265±81	252±77
B (measured at 9 h)	None	6±1	
	50 μM-spermidine	66±3	
	50 μM-spermine	96±14	
	50 μM-N^1N^8-bis(ethyl)spermidine	386±45	
	50 μM-N^1N^9-bis(ethyl)homospermidine	94±15	
	50 μM-N^4-methylspermidine	63±5	
	50 μM-N^4-benzylspermidine	26±5	
	50 μM-N^4-acetylspermidine	26±6	
	50 μM-1,3,6-triaminohexane	40±5	
	50 μM-1,4,7-triaminoheptane	118±30	
	50 μM-deoxyspergualin	31±7	
	5 μM-MGBG	1080±30	
	5 μM-EGBG	220±50	
	5 μM-MBAG	188±80	

The most potent inducing agent found was MGBG. Exposure to 5 μM-MGBG for 9 h gave an increase in SAT which was 16 times greater than the increase with spermidine. The analogues of MGBG, EGBG and MBAG, were also very active compared with the polyamines, but were less powerful than MGBG itself (Table 2). This may be due to their lower uptake into the cell.

More detailed investigations of the induction of SAT by MGBG were carried out and are shown in Fig. 3. The time course of induction by 5 μM-MGBG showed a steady increase in activity, which reached a peak amounting to a more than 400-fold increase after about 50 h of exposure. Pretreatment of the cells with DFMO was not necessary for the induction of SAT by polyamines (results not shown) or by MGBG. However, as shown in Fig. 3, the treatment with DFMO changed the dose–response to MGBG, increasing the extent of induction at low exposures and decreasing it at higher concentrations (Fig. 3). These changes are most likely the result of the enhanced uptake of MGBG in DFMO-pretreated cells (Jänne *et al.*, 1983). The dose–response curves measured after 16 h of exposure showed a maximal effect with 10 μM-MGBG in the control cells and with 4 μM-MGBG in the DFMO-treated cells. At higher concentrations of MGBG the induction declined; this is probably due to the toxic effects of the accumulation of high intracellular concentrations of MGBG.

The effect of MGBG on polyamine contents in the DFMO-treated L6 cells is shown in Fig. 4. There was a substantial increase in putrescine and in N^1-acetylspermidine after exposure to MGBG, and at the same times there was a marked decrease in spermidine and a smaller decrease in spermine. These changes are also consistent with the postulated role of the induced SAT in bringing about the conversion of the higher polyamines into putrescine via the acetylase/oxidase pathway.

The half-life of the SAT induced by spermidine, spermine or MGBG was determined after induction by these agents and the addition of cycloheximide to block protein synthesis (Fig. 5). SAT induced by polyamines had a very short half-life of 9 min after spermidine or 18 min after spermine. MGBG treatment led to a much longer half-life, which could not be measured accurately, since there was little decline over the 2 h period of exposure to cycloheximide. The half-life of SAT in MGBG-treated L6 cells was clearly more than 5 h.

DISCUSSION

We have shown previously that SAT in various rat tissues was induced by administration of polyamines (Pegg & Erwin, 1985). However, in those experiments it was difficult to rule out completely the possibility that the induction is a response to the toxicity associated with this treatment, since a variety of toxic substances also induce SAT in liver (Matsui *et al.*, 1981; Della Ragione & Pegg, 1984; Pegg *et al.*, 1985) and in kidney (Matsui & Pegg, 1982; Pegg *et al.*, 1985). The present experiments show clearly that exposure to exogenous polyamines induces a massive increase in SAT in rat myoblasts before the development of any toxic effects. The induction of SAT

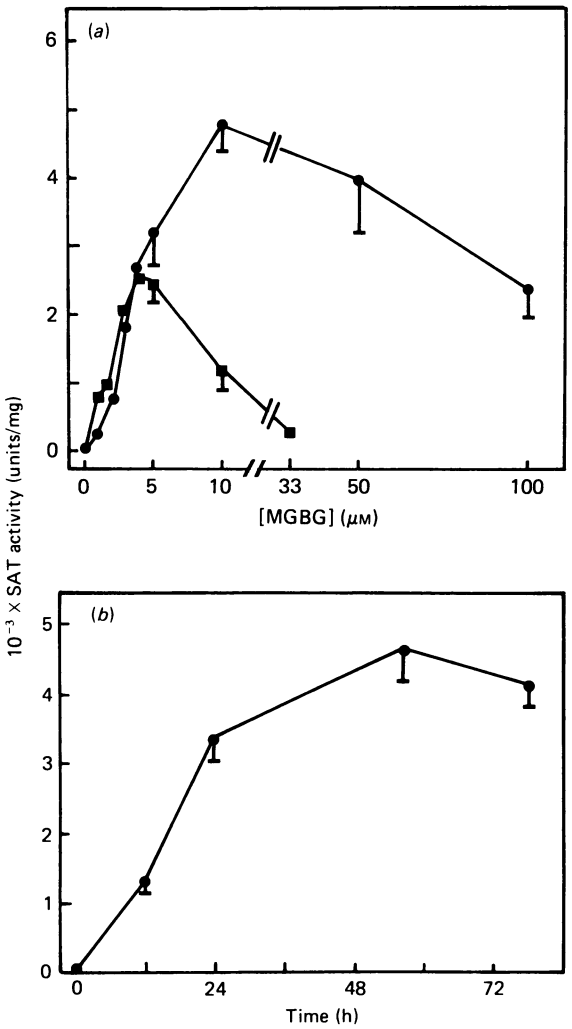

Fig. 3. Effect of MGBG on SAT

Cells were grown in the presence of 1 mM-DFMO (●) as in Table 1 or without DFMO (■), and were then exposed to various concentrations of MGBG as indicated in (*a*) or to 5 μM-MGBG for various times as indicated in (*b*). Extracts were prepared and the SAT activity was determined.

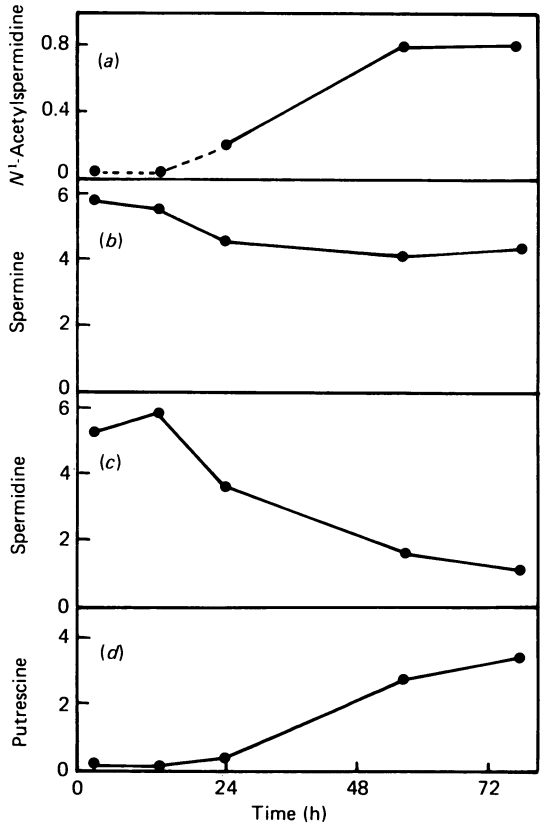

Fig. 4. Effect of MGBG on polyamine content of L6 cells

Cells were grown in the presence of DFMO as in Table 1 and 5 μM-MGBG was then added. At the times shown the contents of N^1-acetylspermidine (*a*), spermine (*b*), spermidine (*c*) and putrescine (*d*) were determined and expressed as nmol/mg of protein in the cells. N^1-Acetylspermine was below the limit of detection (about 0.1 nmol/mg) at all times, and the N^1-acetylspermidine values before 24 h were also below 0.1 nmol/mg.

in response to exogenous polyamines may be a general effect in mammalian cells, since we have found that this increase also occurs in transformed mouse fibroblasts (SV-3T3 cells) and in mouse lymphocytic leukaemia cell lines (L1210 and L5178Y cells) (R. S. Wechter, B. G. Erwin & A. E. Pegg, unpublished work).

SAT was also induced by a wide variety of polyamine analogues (Table 2). A general hypothesis to explain these results would be that an increase in the activity of the acetylase/oxidase pathway for the degradation of the higher polyamines to putrescine occurs in response to an excess in the free intracellular polyamine concentration. The polyamine analogues may bring about this induction of SAT either by mimicking the polyamines directly or by displacing polyamines from intracellular binding sites, which effectively increases the free polyamine concentration. It is possible that induction by toxic agents such as CCl_4, thioacetamide, folic acid and dialkylnitrosamines (Della Ragione & Pegg, 1984) also occurs by release of polyamines into the free pool, owing to destruction of such binding sites.

The increase in SAT clearly leads to the conversion of the higher polyamines, particularly spermidine, into putrescine. It appears that spermidine rather than spermine is the major substrate in the cell for the acetylase/oxidase pathway. These results are in agreement with the observations by Seiler and colleagues, who found that administration of the polyamine oxidase inhibitors produced a much greater accumulation of N^1-acetylspermidine than of N^1-acetylspermine (Seiler *et al.*, 1985; Bolkenius *et al.*, 1985).

Although cellular SAT content was increased to a greater extent by MGBG than by spermidine or spermine, the increase in N^1-acetylspermidine and putrescine in the MGBG-treated cells was delayed until very high SAT contents were reached. A probable explanation for this is that MGBG is not only an inducer but also an inhibitor of SAT (Pegg *et al.*, 1985).

SAT is known to have a very short half-life in rat liver after induction by thioacetamide or polyamines (Persson & Pegg, 1984). It is not possible to measure the half-life of SAT in the uninduced L6 cells, as the activity is so low,

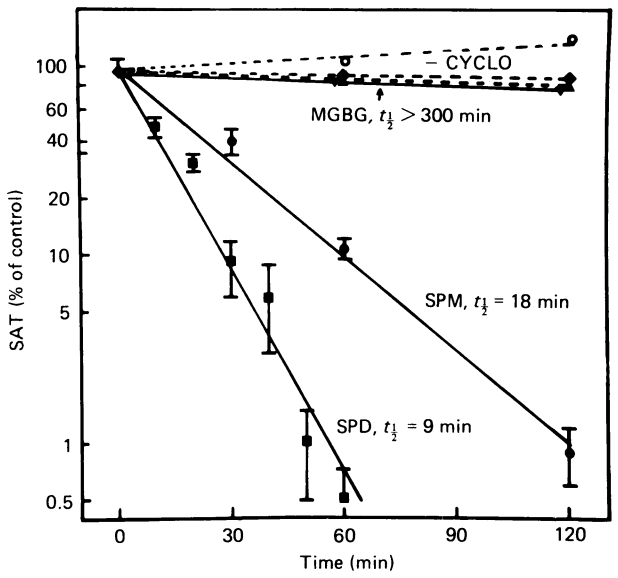

Fig. 5. Half-life of SAT in L6 cells induced by spermidine, spermine or MGBG

L6 cells were grown for 2 days as in Table 1, and SAT was induced by treatment for 6 h with 100 μM-spermidine (SPD; ■ and ◆), 100 μM-spermine (SPM; ● and ○) or 5 μM-MGBG (▼ and ▲). Protein synthesis was then inhibited by the addition of 0.18 mM-cycloheximide (■, ● and ▼) and the decline in SAT activity was measured. Results for cells not treated with cycloheximide (–CYCLO; broken lines; ◆, ○ and ▲) indicated that the SAT activity did not change appreciably over the course of the experiment. The results are expressed as the percentage of the control activity for each treatment before addition of cycloheximide.

but the increase in the amount of SAT which occurs in response to polyamine inducers does not appear to be due to a decrease in its rate of degradation, since the half-life was only 9 min after treatment with spermidine or 18 min after spermine. Therefore, these polyamines must lead to an increased rate of synthesis of the protein. However, at least part of the increase in SAT in response to MGBG is due to the stabilization of the enzyme against intracellular degradation (Karvonen & Pösö, 1984; Pegg *et al.*, 1985; Fig. 5). There are interesting similarities in the regulation of SAT, which is the limiting enzyme in the conversion of the higher polyamines into putrescine, and of ornithine decarboxylase, which is a key enzyme in biosynthesis of putrescine and the polyamines *de novo*. It is well known that ornithine decarboxylase turns over very rapidly and is very highly and rapidly inducible in response to a wide variety of stimuli, leading to increased accumulation of polyamines (Jänne *et al.*, 1978; McCann, 1980; Williams-Ashman & Pegg, 1981; Pegg *et al.*, 1982; Pegg & McCann, 1982; Tabor & Tabor, 1984). Both ornithine decarboxylase and SAT are increased by MGBG, owing to stabilization of the protein against normal degradative processes (Karvonen & Pösö, 1984).

Porter *et al.* (1985) have reported that N^1N^8-bis(ethyl)spermidine has an anti-proliferative effect towards L1210 cells and causes a marked depletion of cellular polyamine pools. They found that N^1N^8-bis(ethyl)spermidine led to a significant decrease in ornithine decarboxylase activity. This may account for the decrease in polyamine content. However, our results showing that N^1N^8-bis(ethyl)spermidine is a good inducer of SAT raise the possibility that part of the decline in polyamine content is due to the induction of the acetylase, followed by either degradation of the acetylated polyamines by polyamine oxidase or their excretion from the cell. Such 'gratuitous' induction by polyamine analogues that are not themselves able to substitute for the normal physiological functions of the polyamines that are essential for cell growth may be an important contributory factor to growth-inhibitory properties of these analogues (Casero *et al.*, 1984; Porter *et al.*, 1985).

This research was supported by grant GM26290 from N.I.H. We thank Dr. J. R. Florini for providing us with the L6 cells and Dr. Lo Persson, who prepared antiserum to SAT.

REFERENCES

Bolkenius, F. N. & Seiler, F. (1981) Int. J. Biochem. **13**, 287–292
Bolkenius, F. N., Bey, P. & Seiler, N. (1985) Biochim. Biophys. Acta **838**, 69–76
Bradford, M. (1976) Anal. Biochem. **72**, 248–254
Casero, R. A., Bergeron, R. J. & Porter, C. W. (1984) J. Cell. Physiol. **121**, 476–482
Danzin, C., Bolkenius, F. N., Claverie, N., Wagner, J. & Grove, J. (1982) Biochem. Biophys. Res. Commun. **109**, 1234–1239
Della Ragione, F. & Pegg, A. E. (1982) Biochemistry **22**, 6152–6158
Della Ragione, F. & Pegg, A. E. (1983) Biochem. J. **213**, 701–706
Della Ragione, F. & Pegg, A. E. (1984) in Advances in Polyamines in Biomedical Science (Caldarera, C. M. & Bachrach, U., eds.), pp. 97–104, CLUEB, Bologna
Erwin, B. G., Persson, L. & Pegg, A. E. (1984) Biochemistry **23**, 4250–4255
Hölttä, E. (1977) Biochemistry **16**, 91–100
Jänne, J., Pösö, H. & Raina, A. (1978) Biochim. Biophys. Acta **473**, 241–293
Jänne, J., Hölttä, E., Kallio, A. & Käpyaho, K. (1983) Spec. Top. Endocrinol. Metab. **5**, 227–293
Karvonen, E. & Pösö, H. (1984) Biochim. Biophys. Acta **791**, 239–243
Matsui, I. & Pegg, A. E. (1982) FEBS Lett. **139**, 205–208
Matsui, I., Wiegand, L. & Pegg, A. E. (1981) J. Biol. Chem. **256**, 2454–2459
Matsui-Yuasa, I., Otani, S., Shu, Z. W. & Morisawa, S. (1984) FEBS Lett. **178**, 297–300
McCann, P. P. (1980) in Polyamines in Biomedical Research (Gaugas, J. M., ed.), pp. 109–124, John Wiley and Sons, New York
Pegg, A. E. (1983) Methods Enzymol. **94**, 239–247
Pegg, A. E. & Erwin, B. G. (1985) Biochem. J. **231**, 285–289
Pegg, A. E. & McCann, P. P. (1982) Am. J. Physiol. **243**, C212–C221
Pegg, A. E., Seely, J. E., Pösö, H., Della Ragione, F. & Zagon, I. S. (1982) Fed. Proc. Fed. Am. Soc. Exp. Biol. **41**, 3065–3072
Pegg, A. E., Erwin, B. G. & Persson, L. (1985) Biochim. Biophys. Acta **842**, 111–118
Persson, L. & Pegg, A. E. (1984) J. Biol. Chem. **259**, 12364–12367
Porter, C. W., Cavanaugh, P. F., Stolowich, N., Ganis, B., Kelly, E. & Bergeron, R. J. (1985) Cancer Res. **45**, 2050–2057
Seidenfeld, J. & Marton, L. J. (1979) Biochem. Biophys. Res. Commun. **86**, 1192–1198
Seiler, N., Bolkenius, F. N. & Rennert, O. M. (1981*a*) Med. Biol. **59**, 334–346

Seiler, N., Bolkenius, F. N., Knödgen, B. & Haegele, K. (1981*b*) Biochim. Biophys. Acta **676**, 1–7
Seiler, N., Bolkenius, F. N. & Knödgen, B. (1985) Biochem. J. **225**, 219–226
Shinki, T., Takahashi, N., Kadofuku, T. & Suda, T. (1985) J. Biol. Chem. **260**, 2185–2190
Stoschek, C. M., Erwin, B. G., Florini, J. R., Richman, R. & Pegg, A. E. (1982) J. Cell. Physiol. **110**, 161–168
Tabor, C. W. & Tabor, H. (1984) Annu. Rev. Biochem. **53**, 749–790
Wallace, H. M., MacGowan, S. H. & Keir, H. M. (1985) Biochem. Soc. Trans. **13**, 329–330
Williams-Ashman, H. G. & Pegg, A. E. (1981) in Polyamines in Biology and Medicine (Morris, D. R. & Marton, L. J., eds.), pp. 44–73, Marcel Dekker, New York
Yaffe, D. (1968) Proc. Natl. Acad. Sci. U.S.A. **61**, 477–483

Received 6 January 1986/2 May 1986; accepted 13 May 1986