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# STRUCTURE–FUNCTION CORRELATIONS OF POLYAMINE ANALOG-INDUCED INCREASES IN SPERMIDINE/SPERMINE ACETYLTRANSFERASE ACTIVITY*

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**Abstract**—The cytosolic enzyme, spermidine/spermine acetyltransferase (SSAT), is distinguished by its role in polyamine interconversion and by its high inducibility in response to a variety of physiological and pharmacological stimuli. Among a series of fifteen polyamines and polyamine analogs, the most potent inducers of SSAT activity in cultured L1210 cells were found to be $N^1$,$N^8$-bis(ethyl)spermidine (BES) and $N^1$,$N^{12}$-bis(ethyl)spermine (BESm). Over a 24-hr exposure at 10 $\mu$M, enzyme activity rose 13- and 16-fold with BES and BESm, respectively, compared to 2- to 3-fold with the anticancer agent, methylglyoxal bis(guanylhydrazone). The increase in enzyme activity by BESm began rapidly and continued steadily with time so that by 48 hr it increased to about twenty times control. By inhibitor studies, the increase was found to be due to elevated protein synthesis predominantly at the level of translation and to an apparent prolongation of enzyme half-life related to enzyme stabilization. Among the analogs, the structural requirements for maximum enzyme induction were found to be critically dependent on aminopropyl moieties and on the presence, size and location of the alkyl groups. By structure–function comparisons, it was deduced that the known abilities of BES and BESm to regulate ornithine and *S*-adenosylmethionine decarboxylase activities or to inhibit cell growth occur independently of their effects on SSAT activity in L1210 cells.

Spermidine/spermine-*N*-acetyltransferase (SSAT‖) is a cytosolic enzyme which catalyzes the transfer of an acetyl group from acetyl coenzyme A to the aminopropyl primary amine of spermidine (SPD) or spermine (SPM). In the case of SPD, which is an asymmetrical molecule, the aminobutyl primary amine can be acetylated by separate acetyltransferases found predominantly in the nucleus [1–3]. Although SPM lacks terminal aminobutyl moieties, it can serve as a substrate for both enzymes.

While the relevance of the nuclear enzyme remains obscure, the cytosolic SSAT has gained prominence for its role in the metabolic interconversion of polyamines [4–6]. The association was realized when Bolkenius and Seiler [7] observed that $N^1$-acetylspermine is an extremely efficient substrate for rat liver cytoplasmic polyamine oxidase (PAO). The latter oxidatively eliminates acetylated aminopropyl units of SPD or SPM by their conversion to 3-acetamido-propanol [8]. Thus, PAO catalyzes the formation of SPD from $N^1$-acetylspermine and putrescine (PUT) from $N^1$-acetylspermidine. From a metabolic perspective, the sequential actions of SSAT and PAO complement the polyamine biosynthetic pathway by allowing for the conversion of higher polyamines back to PUT.

A distinctive feature of cytoplasmic SSAT, which has not been generally noted with the nuclear enzyme, is its high inducibility. The phenomenon was first reported by Matsui and Pegg [9] in livers exposed to carbon tetrachloride and has since been documented to occur in numerous *in vitro* and *in vivo* systems in response to a wide variety of physiological, pathological and pharmacological stimuli [see Ref. 10 for a comprehensive review]. Among the pharmacological stimuli, the antiproliferative agent, methylglyoxal bis(guanylhydrazone) (MGBG) [11], has been shown to be an extremely potent inducer of SSAT activity [12–17]. Studies with cycloheximide indicate that increased new synthesis of SSAT protein is partly responsible for the rise in enzyme activity [15, 16]. In addition, half-life determinations suggest that enzyme stabilization may also contribute to the effect [12, 14, 16].

Recently, Erwin and Pegg [16] reported that, in L6 cells depleted of PUT and SPD by the ornithine

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‖ Abbreviations: BEH, $N^1$,$N^9$-bis(ethyl)homospermidine; BEHSm, $N^1$,$N^{14}$-bis(ethyl)homospermine, actually this is a symmetrical two-carbon homolog of BESm which might be more properly named *N*,$N^1$-bis(4-ethylaminobutyl)-1,4-butanediamine; BEP, $N^1$,$N^4$-bis(ethyl)putrescine; BES, $N^1$,$N^8$-bis(ethyl)spermidine; BESm, $N^1$,$N^{12}$-bis(ethyl)spermine; BMSm, $N^1$,$N^{12}$-bis(methyl)spermine; BPSm, $N^1$,$N^{12}$-bis(propyl)spermine; DESm-4,9, $N^4$,$N^9$-bis(ethyl)spermine; DFMO, $\alpha$-difluoromethylornithine; HEPES, *N*-2-hydroxyethylpiperazine-$N^1$-2-ethanesulfonic acid; MES-4, $N^4$-ethylspermidine; MES-8, $N^8$-ethylspermidine; MGBG, methylglyoxal bis(guanylhydrazone); MOPS, 3-*N*-morpholino propane sulfonic acid; PAO, polyamine oxidase; PBS, phosphate-buffered saline; PUT, putrescine; SPD, spermidine; 6-SPM, 6-spermyne; SPM, spermine; SSAT, spermidine/spermine acetyltransferase; and TES, $N^1$,$N^4$,$N^8$-tris-ethylspermidine.

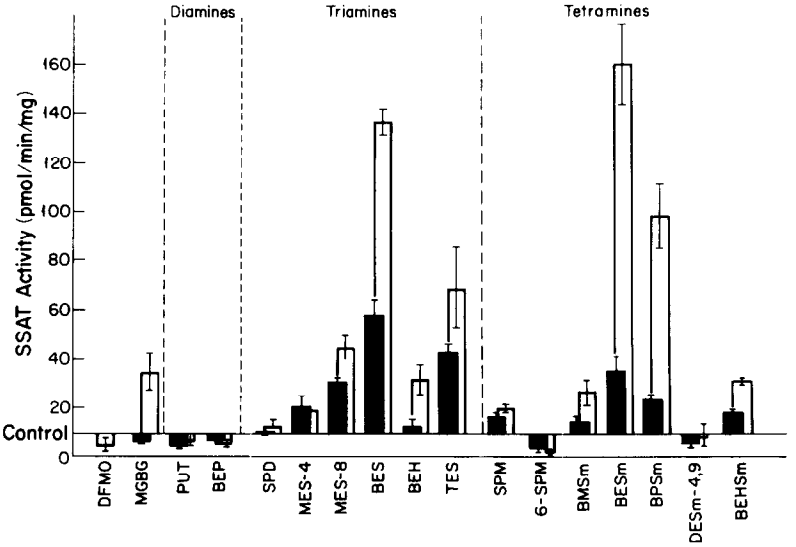


Fig. 1. Induction of SSAT in L1210 cells by 24-hr exposure to 1 $\mu$M (solid columns) or 10 $\mu$M (open columns) of polyamines and analogs. Each column represents the mean value of each of at least two experiments in duplicate with SEM shown on each bar. The control value was 9.27 ± 0.93 (SEM) pmol/min/mg protein.

decarboxylase inhibitor, $\alpha$-difluoromethylornithine (DFMO) [18, 19], the SPD analog, $N^1$,$N^8$-bis(ethyl)-spermidine (BES), produces a very substantial rise in SSAT activity. Since the analog is one in a series of N-alkylated polyamines having significant antiproliferative properties [20, 21], it was of interest to assess the specific effects of these analogs on SSAT activity and to relate these findings, on a structure–function basis, to other known cellular effects of these compounds [21, 22]. We report here that, in L1210 cells not depleted of polyamines, several of the bis(ethyl)polyamine derivatives were much more effective than MGBG in increasing SSAT activity via mechanisms which seem to involve enzyme stabilization and increased enzyme protein synthesis. From structure–function correlations it was deduced that this activity is highly structure dependent and that it is probably not related to the antiproliferative activities of the analogs or to their ability to negatively regulate ornithine and *S*-adenosylmethionine decarboxylase activities [23].

## MATERIALS AND METHODS

Putrescine, spermidine, and spermine were purchased from Sigma; MGBG was from Aldrich, and [1-$^{14}$C]acetyl coenzyme A, 50 mCi/mmol, from New England Nuclear. P-81 phosphocellulose discs, 2.4 cm, were purchased from Whatman. DFMO was a gift from Merrill Dow. Polyamine analogs were synthesized as previously reported [24].

L1210 cells were cultured in RPMI 1640 medium buffered with 16 mM HEPES and 8 mM MOPS to pH 7.4. The medium contained 10% NuSerum IV (Collaborative Research Inc., Bedford, MA) and 1 mM aminoguanidine. Cells were seeded at 17 hr prior to initiation of treatment in 50 ml of medium in T-75 flasks at $4 \times 10^4$ cells/ml unless otherwise stated. At time 0, treatment was started by the addition of drug to the medium. Cells were harvested at various times afterwards, washed once with PBS, suspended at a concentration of approximately $2 \times 10^7$ per ml in 5 mM HEPES, pH 7.2, containing 1 mM dithiothreitol (DTT), and homogenized by brief sonication. The cytosol obtained after a 1-hr centrifugation at 35,000 rpm in a Spinco 40 rotor was used as the source of enzyme for the assay of SSAT activity.

The cytosol was incubated with 10 $\mu$mol HEPES buffer, pH 7.8, 0.15 nmol SPD, and 0.5 nmol [1-$^{14}$C]acetyl coenzyme A, in a final volume of 50 $\mu$l, for 5 min at 37°. The reaction was stopped by chilling, the addition of 20 $\mu$l of 0.5 M $NH_2OH \cdot HCl$, and heating in a boiling water bath for 3 min. After centrifugation to remove precipitated protein, 50 $\mu$l of the reaction was spotted onto a disc of P-81 phosphocellulose and washed and counted as described previously [1]. Protein was measured by the method of Bradford [25], using a commercial preparation purchased from Bio-Rad Laboratories (Richmond, CA). Enzyme activity was expressed as picomoles of acetylspermidine synthesized per minute per milligram of protein.

Intracellular concentrations of BESm were determined as previously reported [26].

## RESULTS

Before presenting observed drug effects on SSAT activity, it is important to indicate that much of SSAT activity measured in control extracts (~10 pmol/min/mg protein) may not be the specific inducible SSAT, but rather due to other acetyltransferases present in the cell. Since Pegg and coworkers [13, 16] have observed, using a specific SSAT antiserum, that most (>90%) of uninduced SAT activity is not

Table 1. Effect of DFMO pretreatment on BESm-induced increases in SSAT activity and uptake in cultured L1210 cells

| Pretreatment (48 hr) | Induction (24 hr) | SSAT activity* (pmol/min/mg protein) | Intracellular BESm† (pmol/$10^6$ cells) |
|---|---|---|---|
| None | None | 9.1 | None |
| 1 mM DFMO | None | 3.6 | None |
| None | 1 $\mu$M BESm | 23.2 | 1.42 ± 0.11 |
| 1 mM DFMO | 1 $\mu$M BESm | 114.5 | 1.57 ± 0.12 |
| None | 10 $\mu$M BESm | 108.5 | 1.83 ± 0.08 |
| 1 mM DFMO | 10 $\mu$M BESm | 150.9 | 1.91 ± 0.18 |

* SSAT values shown are the averages of duplicates which agreed within 15%.
† Values are means ± SEM, N = 4.

specific SSAT, it seems likely that a similar situation may prevail in L1210 cells. Thus, while absolute enzyme activity values for drug-induced SSAT can be considered accurate, expressions of enzyme activity such as percent or fold increases over control probably underestimate the actual effect. The latter, however, are used for convenience of description in reporting drug effects below.

A summary of the effects of the natural polyamines and various polyamine analogs and inhibitors on SSAT activity over a 24-hr period is presented in Fig. 1. Increasing intracellular pools with exogenous polyamines had little effect on SSAT activity with the exception of SPM, which resulted in a minor increase in enzyme activity. These results are in contrast to the large induction of SSAT by spermidine and spermine found in L6 cells [16] and rat liver [27]. Lowering intracellular PUT and SPD levels with 1 mM DFMO [19] also failed to increase SSAT activity. Increases in activity of approximately 2- to 3-fold were achieved, however, with MGBG. These increases are much lower than those reported by Erwin and Pegg [16] in polyamine-depleted cells. Although it is recognized as an inhibitor of AdoMet decarboxylase, MGBG has a number of other biological activities [11] in addition to its ability to act as an SPD analog. Thus, its effects are not likely to be due to decreases in polyamine pools *per se*.

While the polyamines themselves were ineffective in increasing SSAT activity, alkyl derivatives of SPD and SPM, but not PUT, were very active in this regard (Fig. 1). In all cases, the effects were dose dependent so that a 10 $\mu$M concentration usually resulted in greater (but not proportional) increases in enzyme activity than achieved at 1 $\mu$M. Among the triamines, BES was the most effective, yielding a 13-fold increase at 10 $\mu$M. The triethyl derivative, with ethyl groups at all three nitrogens ($N^1$,$N^4$,$N^8$-tris-ethylspermidine, TES) was next active followed by $N^8$-ethylspermidine (MES-8) and $N^8$-ethylspermidine (MES-4). Interestingly, $N^1$,$N^9$-bis(ethyl)homospermidine, which differs from BES by having an aminobutyl moiety in place of the aminopropyl group was among the least effective of the triamines, increasing activity only 2- to 3-fold at 10 $\mu$M as compared to 13-fold with BES.

Since Erwin and Pegg [16] reported that, in polyamine-depleted L6 cells, MGBG is more effective than BES, experiments were carried out with cells that had been maintained for 48 hr in 1 mM DFMO prior to treatment. However, BESm was used instead of BES or MGBG for these studies since it had been found to be the most potent enzyme inducer. Table 1 shows that, under conditions of PUT and SPD depletion, cells were more responsive to BESm than cells with normal polyamine pools. At 1 $\mu$M, BESm caused only a small increase in SSAT activity in control cells, but in DFMO-treated cells the increase was greater than that achieved with 10 $\mu$M BESm in untreated cells. The intracellular levels of BESm indicate that the DFMO enhancement of SSAT induction was probably not related to increased analog uptake since, at 1 $\mu$M BESm in particular, DFMO pretreated cells contained only slightly more BESm than non-pretreated cells yet their enzyme activity was about nine times higher (Table 1) than non-pretreated cells. The inconsistency between enzyme activity and intracellular BESm was less apparent in cells treated with 10 $\mu$M BESm. Thus, the basis for DFMO enhancement of BESm induction of SSAT activity does not seem to be related to increased intracellular BESm accumulation unless it involves kinetics of BESm uptake, which were not determined.

Compounds of the tetramine series were even more effective than the comparable triamines and exhibited similar structural specificity for modulating SSAT activity (Fig 1). As in the triamine series, the N-terminal bis(ethyl) derivative [$N^1$,$N^{12}$-bis-(ethyl)spermine, BESm] was the most active and resulted in a 16-fold increase in enzyme activity at 10 $\mu$M. Replacing the ethyl groups with propyl groups [$N^1$,$N^{12}$-bis(propyl)spermine, BPSm] compromised enzyme activity somewhat but not as significantly as replacing them with methyl groups [$N^1$,$N^{12}$-bis(methyl)spermine, BMSm] which diminished activity to levels comparable to SPM. The effects of BESm are apparently dependent on position as well as size of the alkyl moieties since $N^4$,$N^9$-diethylspermine (DES-4,9) increased enzyme activity by only about 2-fold. Enzyme induction was also apparently stringently controlled by the presence of aminopropyl moieties in BESm. Their replacement with aminobutyl groups, as demonstrated with $N^1$,$N^{14}$-bis(ethyl)homospermine (BEHSm), profoundly reduced enzyme enhance-

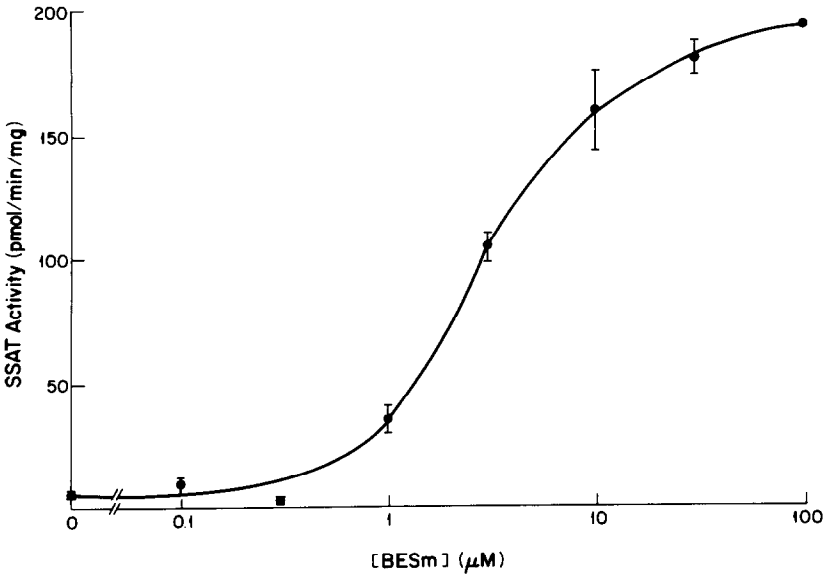


Fig. 2. Concentration response of SSAT activity to increasing levels of BESm for 24 hr. Values are means ± SD, N = 0.

ment as was also observed with SPD analogs. In similarity to SPM, itself, the unsaturated analog, 6-spermyne, failed to increase SSAT activity by more than 2- to 3-fold.

The increase in enzyme activity by the most potent of the compounds tested, BESm, was characterized further with respect to dose and time dependence. A concentration–response curve at 24 hr is shown in Fig. 2. Enzyme activity first responded by increasing at 1 μM BESm and then leveled at 30–100 μM after increasing by about 20-fold over control. Thus, maximum induction was achieved at about 100 μM BESm and half-maximal induction at 2.5 μM. The time–course development of the phenomenon was compared for 10 μM BES and BESm over a 48-hr period (Fig. 3). Enzyme activity increased continuously during the entire 48 hr with BESm but, unexpectedly, reached a maximum at 18 hr with BES and decreased slowly thereafter.

The initial finding by Karvonen and Poso [12] that MGBG both induces and stabilizes SSAT activity in rat liver has been confirmed by a number of investigators [14–17]. Accordingly, the effects of BESm and MGBG on the half-life of the enzyme were compared in L1210 cells. In the presence of cycloheximide, the $T_{1/2}$ for BESm-induced enzyme was found to be 72 min and that for MGBG, >120 min (Fig. 4). Because of the low level of control SSAT activity, it was not possible to accurately measure the half-life of SSAT in untreated cells by the enzyme activity assay. However, Erwin and Pegg [16] have found that the enzyme protein half-life of spermine-treated L6 cells is about 9 min. We expect that it may be similarly short in L1210 cells.

To determine whether the increase in enzyme activity may also be dependent on enhanced protein synthesis, the effects of cycloheximide and actinomycin D on enzyme induction were examined. Table 2 presents the results of one experiment in which cells were treated with BESm in the presence of cycloheximide or actinomycin D for a period of 6 hr. Cycloheximide treatment completely abolished the increase in SSAT activity with BESm treatment, suggesting that it was due to new protein synthesis.

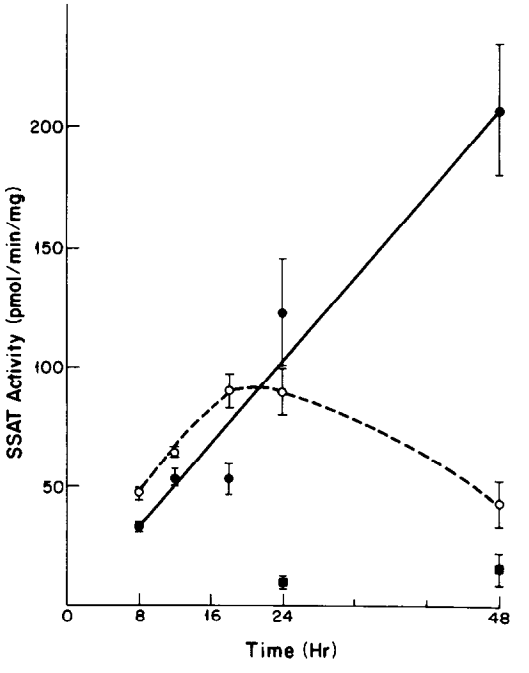


Fig. 3. Time course of SSAT induction of L1210 cells treated with 2 μM BES or 2 μM BESm. Key: (●——●) BESm; (○---○) BES; and (■) control. Values are means ± SD, N = 4.

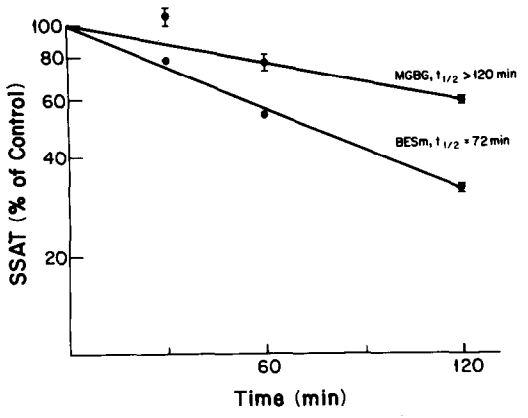


Fig. 4. Half-life determination of SSAT activity in L1210 cells exposed to 2 μM BESm or 10 μM MGBG for 24 hr. Following drug exposure, cells were then treated with 0.18 mM cycloheximide and assayed for enzyme activity. Control values for the BESm-induced cells and the MGBG-induced cells were 70.9 and 82.6 pmol/min/mg protein respectively. Values are means ± SD, N = 4.

By contrast, actinomycin D (at a level sufficient to inhibit the incorporation of uridine into macromolecules by greater than 93%), restricted the rise in SSAT activity during BESm treatment to only 50%. The data suggest that control of the enzyme protein synthesis was exerted primarily at the level of protein translation and, to a lesser extent, gene transcription.

Finally, we have previously provided evidence for the identity of nuclear spermidine acetyltransferase activity and nuclear histone acetyltransferase activity [1]. Matsui *et al.* [28] have demonstrated that the induced SSAT activity of rat liver has no activity toward histones. We examined the histone acetyltransferase activity of L1210 cytosols from control and BESm-treated cells and found no increase in histone acetylation (data not shown) and thus conclude that, in this system also, SSAT is distinct from the histone acetyltransferase activity.

## DISCUSSION

As summarized in Fig. 1, the relative abilities of various polyamines and polyamine analogs to increase SSAT activity suggest that strict structural constraints may be involved. The definition of these relationships, however, must be made with recognition that, in addition to analog interaction at critical binding site(s) related to the enzyme induction, cellular uptake could also be involved. Thus, except in cases where relative uptake is known, the structure–function correlations described below apply to their overall effects on whole cells and could be related to one or both of the above factors.

Of the various structural correlates examined, enzyme induction seemed to be most obviously dependent on the presence of N-terminal alkyl groups. While SPD or SPM resulted in only minor increases in enzyme activity, their alkylated analogs, BES and BESm, gave the greatest increases of any compounds tested including MGBG. This effect cannot be attributed to drug uptake since we have shown previously [26] that both BES and SPD enter cells at similar rates, as indicated by their nearly identical abilities to negatively regulate ornithine decarboxylase activity.

The greater effectiveness of BES over MGBG in increasing SSAT activity differs from the previous findings of Erwin and Pegg [16] who reported that, in L6 cells, MGBG was more potent than BESm. In those experiments, however, the cells were treated with DFMO prior to drug exposure in order to deplete intracellular polyamine pools. In the L1210 cell system at least, DFMO pretreatment enhanced SSAT induction by BESm, and this may be due to increased uptake of the analog. Since uptake of MGBG is also known to increase as a result of DFMO-induced polyamine depletion [29], this may contribute, in part, to the variance in findings. In addition, differences in cell types and in treatment conditions are also likely to play a role.

SSAT induction was also apparently dependent on the size and location of the alkyl groups. Changing the terminal ethyl groups of BES to methyl or propyl groups as demonstrated by BMSm or BPSm, respectively, diminished enzyme induction, especially in the case of BMSm which was comparable to SPM in effectiveness. Likewise, when the ethyl groups were affixed to the internal nitrogens such as in the case of MES-4 or DESm-4,9 instead of at the terminal nitrogens, enzyme induction was reduced markedly compared to BES or BESm.

The presence of alkylaminopropyl moieties was found to be a critical factor with respect to the internal aliphatic chain length specificity of the SSAT effect. This was independently demonstrated with three bis(ethyl) derivatives, BEP, BEH and BEHSm, all of which lacked alkylaminopropyl

Table 2. Effect of actinomycin D and cycloheximide on induction of SSAT activity by the spermine analog BESm

| Treatment (6 hr) | SSAT activity* (pmol/min/mg protein) |
|---|---|
| Control | 7.9 |
| 10 μM BESm | 33.2 |
| 10 μM BESm + 4 μM actinomycin D | 17.1 |
| 10 μM BESm + 0.18 mM cycloheximide | 8.2 |

* Values are the averages of duplicates which agreed within 10%.

Table 3. Comparison of growth inhibition, decarboxylase regulation and SSAT induction among polyamines and polyamine analogs in cultured L1210 cells

| Polyamine or analog* | $IC_{50}$ ($\mu$M) | Regulation of activity† | | Induction of SSAT activity† |
|---|---|---|---|---|
| | | ODC | AdoMetDC | |
| PUT‡ | >1000 | ± | − | − |
| BEP‡ | >1000 | ± | − | − |
| SPD‡ | >1000 | ++ | + | − |
| MES-4§ | >1000 | ± | − | ± |
| MES-8‖ | 100 | + | ± | ++ |
| BES‡ | 50 | ++ | ± | ++ |
| BEH‖ | 300 | ++ | − | ± |
| TES‖ | 300 | ± | − | ++ |
| SPM‡ | 160 | ±± | ++ | ± |
| BMSm‖ | 100 | ++ | ++ | ± |
| BESm‡ | 10 | ++ | ++ | ++ |
| BEHSm‖ | 1 | ++ | ++ | ± |
| BPSm‖ | 3 | ++ | ++ | ++ |
| DESm-4,9‖ | 400 | − | − | − |
| 6-SPM¶ | >100 | ± | ++ | − |

* At 1–10 $\mu$M for 24 hr.
† Scale: ++, very strong; +, definite, ±, weak; −, none.
‡ $IC_{50}$, ODC, and AdoMetDC data taken from [26].
§ $OD_{50}$, ODC, and AdoMetDC data taken from [22].
‖ Unpublished observations.
¶ $IC_{50}$, ODC, and AdoMetDC taken from [35].

moieties and all of which were ineffective in increasing SSAT activity. The finding is consistent with the known substrate specificity of the enzyme for aminopropyl moieties [30], suggesting that enzyme active site binding may occur with BES and BESm and this, in turn, could result in enzyme stabilization. A possible exception to the above correlation is apparent in the ability of the SPD analog with an ethyl group at the aminobutyl primary amine (MES-8) to increase SSAT activity by 3- to 4-fold. The possible basis for this anomaly may reside in differences in cellular uptake between the analogs.

Several reports have appeared which show that MGBG stabilizes SSAT, and that this effect accounts in part for the increase in activity seen after MGBG treatment [12, 15–17]. As shown in Fig. 4, MGBG indeed prolonged the half-life to 120 min—almost twice that of the enzyme induced by BESm (72 min). Because SSAT activity in untreated L1210 cells is too low to establish half-life determinations by enzyme assay, we cannot be definitive about the significance of the above values. However, in two other systems, rat liver and L6 cells, half-lives as short as 9 min have been reported for SSAT [16]. Although we have no evidence for it, L1210 SSAT probably has a half-life comparable to these values, since other L1210 polyamine metabolic enzymes show similarities to those of other cell systems [21, 31]. Under this assumption, the 72-min half-life of BESm-induced SSAT would seem to represent an appreciable stabilization of the enzyme by the analog.

While enzyme stabilizaton may account, in part, for the analog-induced increases in SSAT activity, other mechanisms also seem to be involved. Cycloheximide experiments indicate that protein synthesis is necessary since, in the presence of the drug, BESm-mediated increases in SSAT activity were abolished completely. Inhibition of RNA synthesis with actinomycin D, however, allowed for a 50% induction in enzyme activity, suggesting that there may be an excess of SSAT messenger RNA and that the control of enzyme protein synthesis may be only partially exerted at the levels of gene transcription. The predominant effect, however, would seem to be at the level of protein translation.

In similarity to these findings, a number of investigators [15, 32–34] have reported the abolition of stimulated SSAT activity with concurrent administration of cycloheximide or actinomycin D. It is interesting that Shinki *et al.* [33] also found that, while cycloheximide completely prevents the 1$\alpha$,25-dihydroxyvitamin $D_3$ stimulated rise in SSAT activity in chick duodenum, actinomycin D is only partially effective in preventing the increase in enzyme activity.

Because induction of SSAT activity by BES or BESm appears to be a regulatory response that occurs with a rapidity resembling analog-mediated regulation of ornithine and *S*-adenosylmethione decarboxylase activities [21, 23] and since both seem to occur at the level of enzyme translation albeit in opposite directions, the two events could conceivably be causally related. By structure–function correlations, we were able to discount this possibility. Table 3 compares effects of these compounds on growth, ornithine decarboxylase and AdoMet decarboxylase with effects on SSAT. Both SPD and SPM strongly regulate the decarboxylase activities but produce only minor increases in SSAT activity. Of the alkyl derivatives, 6-spermyne strongly regulates *S*-adenosylmethionine decarboxylase [35] but is a weak inducer of SSAT activity. Conversely, TES

is a potent inducer of SSAT activity but has only minimal effects on decarboxylase activities (Porter and Bergeron, unpublished data). Since, unlike previous structure–function correlations, these deductions are not complicated by differences in analog uptake (i.e. BES and BESm accumulate in cells at rates similar to SPD and SPM [21]), it may be unambiguously concluded that the two regulatory events occur independently of one another.

Although not yet proven definitively, compelling evidence suggests that, in L1210 cells, growth inhibition by the bis(ethyl) analogs is causally linked to polyamine depletion [20, 26]. Conceivably, then, an upward shift in polyamine catabolism resulting from analog-induced increases in SSAT activity could contribute to depletion of cellular pools and, hence, to the antiproliferative properties of the analogs. This, however, seems not to be the case since BEHSm produces only minor increases in SSAT activity but has potent growth inhibitory effects which, like BESm, seems to be mediated by interference with polyamine biosynthesis (Table 3). While in L1210 cells, enzyme induction is apparently unrelated to *in vitro* growth inhibition by the analogs, it could influence their *in vivo* activity. If, for example, BESm or BES is partially N-dealkylated in the liver, the elevated levels of SSAT in tumor tissues could contribute to the further catabolism of the analog and, hence, diminish its antitumor activity. Thus, analogs which fail to induce SSAT activity could be more effective antitumor agents *in vivo* than those which do. Comparative studies with BESm and BEHSm will obviously be useful in dissecting this possibility and in determining whether induction of SSAT activity may play a role in the antiproliferative response of cell lines other than L1210 cells to these analogs.

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