Independent Regulation of Ornithine Decarboxylase and S-Adenosylmethionine Decarboxylase in Methylthioadenosine Phosphorylase-deficient Malignant Murine Lymphoblasts

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ABSTRACT

The control of polyamine synthesis in neoplastic cells is complex and incompletely understood. Using murine lymphoma cells deficient in methylthioadenosine (MTA) phosphorylase, we have analyzed the role of MTA in the regulation of ornithine decarboxylase and S-adenosylmethionine (SAM) decarboxylase, the two rate-limiting enzymes in the polyamine-biosynthetic pathway. The addition of MTA to the enzyme-deficient lymphoblasts induced within 1 to 3 h an increase in the activities of both decarboxylases and an accompanying rise in putrescine and decarboxylated SAM levels. The ornithine decarboxylase inhibitor α-difluoromethylomithine blocked the MTA-triggered accumulation of putrescine but not decarboxylated SAM. In a reciprocal manner, the SAM decarboxylase inhibitor methylglyoxal bis(guanylhydrazone) prevented the acretion of decarboxylated SAM but not putrescine. The MTA-induced rise in SAM decarboxylase and ornithine decarboxylase activities preceded by several hours changes in spermidine or spermine pools. However, MTA decreased the flux through the polyamine-synthetic pathway, as estimated by the incorporation of radioactive ornithine into spermine. Similar changes in polyamine metabolism were observed in a secondary mutant deficient in MTA phosphorylase, but resistant to MTA toxicity. These results suggest that the velocity of polyamine synthesis, or the concentration of MTA itself, may regulate ornithine decarboxylase and SAM decarboxylase activities through separate, growth-independent mechanisms.

INTRODUCTION

Polyamines are organic cations that subserve diverse functions in dividing mammalian cells. States of increased cellular proliferation, such as cancer, are commonly associated with accelerated rates of polyamine synthesis. The factors regulating polyamine synthesis and degradation in normal and malignant cells have been investigated in several laboratories but have not been definitively elucidated (1–6).

MTA3 is generated stoichiometrically from S-adenosyl-(5')-deoxy-(5')-3-methylthiopropylamine (commonly called decarboxylated SAM) during the synthesis of the polyamines spermidine and spermine. Normally, mammalian cells cleave the thioether nucleoside via a specific enzyme, MTA phosphorylase. The 2 reaction products, adenine and 5-methylthioribose-1-phosphate, are reconverted to adenine nucleotides (7) and methionine (8, 9), respectively. In 1977, Tooye (10) reported the absence of MTA phosphorylase in 4 murine malignant hematological cell lines. Subsequent experiments from this laboratory (11, 12) demonstrated that MTA phosphorylase activity was lacking in many human and murine malignant cell lines of diverse origin. Importantly, some leukemic cell clones obtained directly from patients were also phosphorylase deficient (13). In contrast, MTA phosphorylase was abundant in all normal human tissues and in cell lines established from them.

MTA phosphorylase deficiency represents an "experiment of nature" that distinguishes some malignant cell lines from their normal counterparts. Understanding the effects of MTA on cellular metabolism could provide insight concerning the overall control of polyamine synthesis in normal and neoplastic states. In the present work, we have analyzed the time-dependent changes in SAM and polyamine metabolism elicited by MTA in an enzyme-deficient malignant lymphoblastoid cell line (R1.1H). In dividing cell cultures, MTA rapidly and dose dependently augmented the activities of ornithine decarboxylase and SAM decarboxylase, the 2 rate-limiting enzymes in the polyamine-synthetic pathway. The rise in the activities of the 2 decarboxylases in turn caused a progressive accumulation of putrescine and decarboxylated SAM within the cells. The MTA-induced changes occurred prior to any observable alterations in intracellular polyamine pools, or in cell growth, but were accompanied by a decrease in the rate of spermine biosynthesis. These results suggest that ornithine decarboxylase and SAM decarboxylase in malignant cells may be regulated by separate mechanisms that are not solely dependent on the state of cellular proliferation. Portions of this work have been communicated in a preliminary form (14).

MATERIALS AND METHODS

Cell Lines. The murine T-lymphoma cell line, R1.1, came from Dr. Robert Hyman (Salk Institute, La Jolla, CA). A mutant (clone H) deficient in MTA phosphorylase was selected from wild-type R1.1 cells by a "tritium suicide" method, as described previously (15). A secondary mutant clone (H5) was isolated by limiting dilution after clone H cells were cultured for several months in medium containing increasing MTA concentrations (from 10 μM to 1 mM). The H5 cell line proliferated equivalently, whether or not 500 μM MTA was included in the medium. In metabolic studies, the cells were dispersed in RPMI-1640 medium supplemented with 10% heat-inactivated horse serum or with 9% heat-inactivated horse serum, 1% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 2 mm L-glutamine, all from Microbiological

1This work is supported by Grants CA31497 and CA35048 from the National Cancer Institute, and by Grant GM23200 from the NIH. This is Publication 3750BCR from the Research Institute of Scripps Clinic, La Jolla, CA 92037.
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3The abbreviations used are: MTA, 5'-methylthioadenosine; SAM, S-adenosylmethionine; DFMO, DL-α-difluoromethylomithine; MGBG, methylglyoxal bis(guanylhydrazone).
4Received 1/30/85; revised 4/25/85; accepted 5/1/85.
RESULTS

MTA-induced Changes in Polyamine Synthesis and Intracellular Polyamine Pools. Although MTA is a potent inhibitor of polyamine synthesis, its rapid degradation by MTA phosphorylase prevents unambiguous evaluation of its possible role in the regulation of this pathway. We isolated a MTA phosphorylase-deficient clone (H) containing less than 1% of wild-type activity. All other investigated enzyme activities related to purine and polyamine metabolism were comparable to wild type (15). In the mutant, the intracellular MTA levels reflected the added MTA concentrations in the medium (data not shown). The uptake mechanism for MTA is probably facilitated diffusion (22). The intracellular degradation of MTA in the mutants has been shown to be insignificant (22). The addition of MTA to the medium of proliferating MTA phosphorylase-deficient murine lymphoma cells induced a rapid and progressive rise in the activities of SAM decarboxylase and ornithine decarboxylase. As shown in Chart 1, SAM decarboxylase activity doubled after 1 h and had increased 8-fold by 6 h after the addition of 0.5 mM MTA. The activity of ornithine decarboxylase rose significantly by 2 h after the addition of MTA and thereafter increased in parallel with SAM decarboxylase.

During the first 6 h after the addition of MTA, the enzyme-deficient lymphoblasts continued to proliferate at the same rate as untreated cells, as estimated by direct cell counting, and by radioactive thymidine uptake at 2-h intervals (results not shown). The very early effects of MTA on polyamine metabolism were not a nonspecific consequence of alterations in cell growth kinetics. Thus, the same changes occurred in clone H5, that grew normally in 500 µM MTA.

The increased activity of SAM decarboxylase was accompanied by a concomitant rise in decarboxylated SAM pools. As illustrated in Chart 2, decarboxylated SAM levels rose from undetectable levels (<1 pmol/10^6 cells) in untreated lymphoma cells to 20 pmol/10^6 cells at 1 h. By 3 h after the addition of MTA, decarboxylated SAM and SAM pools were nearly equivalent. These changes were similar in all R1.1 sublines. In a

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Effect of MTA on dcSAM Levels

Chart 2. MTA-induced changes in decarboxylated SAM (dcSAM) levels. R1.1 wild type (Δ and Δ), MTA phosphorylase-deficient clone H (C and O), and MTA phosphorylase-deficient, MTA-resistant clone H5 (□ and ◦) cells were incubated with 500 μM MTA as described in Chart 1. The intracellular contents of decarboxylated SAM (solid symbols) and the ratio of decarboxylated SAM to SAM (open symbols) were determined after separation by high-performance liquid chromatography as described in "Materials and Methods." This experiment was repeated 3 times with similar results.

 complementary fashion, intracellular putrescine content changed in concert with the activity of ornithine decarboxylase. Putrescine content increased to 150% of control by 3 h and was elevated 3-fold at 5 h, as shown by the data of Chart 3 for clone H cells. By contrast, short-term exposure to MTA did not alter intracellular spermidine or spermine levels (Chart 3). Nonetheless, the MTA treatment did inhibit spermine biosynthesis, as estimated by the incorporation of radioactive ornithine into spermine (Chart 4). MTA did not block severely ornithine incorporation into spermidine. Supplementation of the culture medium with spermidine could partially prevent MTA effects. When spermidine was added at 100 μM simultaneously with 0.5 mM MTA, ornithine decarboxylase activity dropped to unmeasurable levels by 1 h. SAM decarboxylase activity initially rose slightly (130% of control activity at 2 h), but it fell to 71% of control by 4 h. At this latter time point, the decarboxylated SAM:SAM ratio was 0.15, compared to 1.0 in MTA-supplemented medium lacking exogenous spermidine.

Table 1 summarizes the dose-related effects of 16-h exposure to exogenous MTA on the levels of polyamines, SAM, and decarboxylated SAM in the MTA phosphorylase-negative as well as -positive lymphoma cells. As little as 10 μM of the thioether nucleoside elevated putrescine and decarboxylated SAM levels. Consistent with the MTA-mediated block in de novo spermine formation, 16-h incubation with the nucleoside dose dependently reduced intracellular spermine concentrations.

Effect of Inhibitors of Polyamine Synthesis. DFMO is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase (23). MGBG is a competitive inhibitor of SAM decarboxylase (24). The inclusion of DFMO in the culture medium blocked the

MTA-induced increase in putrescine pools but further enhanced the elevation in decarboxylated SAM levels (Table 2). In a reciprocal manner, MGBG inhibited the MTA-induced rise in decar-
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Boxylated SAM levels but did not prevent the increase in putrescine pools. These data indicate that the effects of MTA on putrescine and decarboxylated SAM concentrations occurred independently and that the rise in putrescine and decarboxylated SAM levels depended upon the ongoing activity of ornithine decarboxylase and SAM decarboxylase, respectively.

DISCUSSION

The addition of MTA to cultured lymphoma cells severely perturbed SAM and polyamine metabolism. The earliest changes induced by MTA were (a) at 1 h, an increase in SAM decarboxylase activity and a concomitant rise in decarboxylated SAM pools and (b) at 2 to 3 h, an elevation in ornithine decarboxylase activity and a secondary expansion of putrescine pools. The activities of the 2 decarboxylases continued to increase for at least 6 h in MTA-supplemented medium. At that time, decarboxylated SAM levels were equivalent to, or even greater than, total SAM concentrations. Importantly, these metabolic changes occurred independently of detectable alterations in spermidine or spermine content and several h before cell growth declined. These results establish (a) that MTA is a rapid inducer of SAM decarboxylase and ornithine decarboxylase in the enzyme-deficient lymphoma cells, (b) that the stimulation of enzyme activity is one cause for the MTA-induced increase in putrescine and decarboxylated SAM pools, and (c) that these effects are not mediated by a product of MTA catabolism.

The regulation of putrescine and polyamine synthesis in mammalian cells is exceedingly complex and has been reviewed (4, 6). Both ornithine decarboxylase and SAM decarboxylase are minimally active in nonproliferating cells but are induced rapidly after application of a growth-promoting stimulus. In dividing cells, the half-lives of the 2 enzymes are extremely short. It has been pointed out that, when general protein synthesis increases, enzymes that have a high rate of synthesis and degradation respond with a greater and more rapid rise in activity than do enzymes with a slow turnover rate (6). The rapid induction of the 2 enzymes by MTA distinguishes the regulation of the 2 decarboxylase activities from nonspecific changes in growth kinetics. MTA is a cytostatic agent toward normal and malignant lymphocytes, despite its ability to augment ornithine decarboxylase and SAM decarboxylase activities. Importantly, MTA exerted similar effects on decarboxylated SAM and polyamine synthesis in lymphoma cells sensitive or resistant to the antiproliferative effects of the nucleoside.

In cultured cells, the functional activities of ornithine decarboxylase and SAM decarboxylase reflect a balance between the synthetic and degradative rates of the 2 enzymes and their respective states of activation (6, 25–27). The content of putrescine and polyamines may influence one or several of these parameters. In model systems, the addition of spermidine to growing cells has been shown to reduce ornithine decarboxylase and SAM decarboxylase activities (25, 28, 29). In the present studies, exogenous spermidine prevented the MTA-induced changes in the 2 decarboxylases and the associated increase in putrescine and decarboxylated SAM levels. Thus, the MTA treatment did not preclude the negative regulation by spermidine on putrescine and decarboxylated SAM synthesis.

Table 1

MTA-induced changes in SAM and polyamine metabolism in MTA phosphorylase-positive and -deficient lymphoma cells

MTA phosphorylase-deficient R1.1 lymphoma cells (clone H), secondary mutant cells resistant to MTA (H5), and MTA phosphorylase-positive wild-type cells were incubated for 16 h with the indicated concentrations of MTA, and the intracellular contents of polyamines, SAM, and decarboxylated SAM were determined.

<table>
<thead>
<tr>
<th>Polyamines (mmol/10⁶ cells)</th>
<th>SAM (mmol/10⁶ cells)</th>
<th>Decarboxylated SAM (mmol/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putrescine</td>
<td>Spermidine</td>
<td>Spermine</td>
</tr>
<tr>
<td>None</td>
<td>0.46 ± 0.06</td>
<td>2.79 ± 0.17</td>
</tr>
<tr>
<td>MTA, 10 μM</td>
<td>0.84 ± 0.03</td>
<td>2.73 ± 0.11</td>
</tr>
<tr>
<td>MTA, 100 μM</td>
<td>1.60 ± 0.20</td>
<td>2.63 ± 0.24</td>
</tr>
<tr>
<td>MTA, 500 μM</td>
<td>1.60 ± 0.33</td>
<td>2.30 ± 0.21</td>
</tr>
<tr>
<td>None</td>
<td>0.45 ± 0.04</td>
<td>2.80 ± 0.25</td>
</tr>
<tr>
<td>MTA, 100 μM</td>
<td>1.57 ± 0.71</td>
<td>2.66 ± 0.32</td>
</tr>
<tr>
<td>MTA, 500 μM</td>
<td>1.62 ± 0.16</td>
<td>1.47 ± 0.16</td>
</tr>
<tr>
<td>None</td>
<td>0.50 ± 0.02</td>
<td>2.58 ± 0.41</td>
</tr>
<tr>
<td>MTA, 100 μM</td>
<td>1.00 ± 0.04</td>
<td>2.45 ± 0.38</td>
</tr>
<tr>
<td>MTA, 500 μM</td>
<td>1.43 ± 0.03</td>
<td>2.12 ± 0.46</td>
</tr>
</tbody>
</table>

* Mean ± SD of 3 experiments.

* Mean of 2 to 3 experiments.

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MTA is a powerful inhibitor of mammalian spermine synthase (30–31) and, to a lesser degree, of spermidine synthase (32). In the MTA phosphorylase-deficient lymphoma cells, MTA did not substantially reduce spermidine and spermine levels, at least during short-term incubations. However, the thioether nucleoside did block the incorporation of radioactive ornithine into spermine, suggesting that MTA did inhibit spermine synthase activity. The net rate of spermine formation reflects both its synthesis and degradation (4). Possibly, the addition of MTA was followed by a reduction in spermine catabolism. This may explain why, in short-term experiments, no fall of spermine levels was evident, in spite of the greatly reduced synthetic rate. The MTA-induced augmentation of putrescine and polyamine levels may be attributed not only to an increase in ornithine decarboxylase and SAM decarboxylase activity but also to a lack of their utilization for spermine synthesis.

Putrescine has been shown to stimulate SAM decarboxylase activity by stabilizing the enzyme and by lowering the $K_m$ for SAM (33). The rise in decarboxylase activity induced by MTA must be largely independent of this mechanism. Thus, SAM decarboxylase activity increased prior to any observable changes in putrescine pools. Furthermore, the ornithine decarboxylase inhibitor DFMO prevented the MTA-triggered changes in putrescine levels, without altering the effects of the nucleoside on decarboxylated SAM levels.

The exact mechanism by which MTA enhances ornithine decarboxylase and SAM decarboxylase activities remains unclear. The data suggest that nonspecific effects on protein synthesis, as well as alterations in intracellular polyamine pools, do not play an important role. Nonetheless, MTA did inhibit the flux through the polyamine-biosynthetic pathway. Conceivably, ornithine decarboxylase and SAM decarboxylase activities are regulated by the velocity of polyamine synthesis, rather than by the intracellular concentrations of polyamines.

Pegg et al. (34) and Raina et al. (35) have demonstrated that the nondegradable MTA analogue methylthiotubercidin elevated ornithine decarboxylase and SAM decarboxylase activities in cultured cells. Methylthiotubercidin is a substantially weaker inhibitor than MTA of spermine formation (35). It is possible that MTA, and also methylthiotubercidin, directly affects the synthesis, state of activation, or degradation of ornithine decarboxylase and SAM decarboxylase. A direct analysis of the effects of MTA on the generation and turnover of mRNA species corresponding to the 2 enzymes should clarify these issues.

Collectively, our experiments indicate that MTA is a rapid and potent inducer of SAM decarboxylase and ornithine decarboxylase in MTA phosphorylase-negative lymphoma cells. The increased activity of the 2 decarboxylases, combined with a reduction in polyamine synthesis, causes a progressive rise in decarboxylated SAM and putrescine levels. The MTA-triggered rise in SAM decarboxylase and ornithine decarboxylase activities cannot be attributed to alterations in spermidine or spermine levels, nor to changes in the rate of cellular proliferation. These results support the notion that MTA, the nucleoside by-product of polyamine biosynthesis, may also influence the rate-limiting enzymes in the polyamine-synthetic pathway.
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