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ORNITHINE DECARBOXYLASE AND SPERMIDINE/SPERMINE N^1-ACETYLTRANSFERASE ARE INDUCED IN K562 CELLS BY *S*-ADENOSYLMETHIONINE DECARBOXYLASE INHIBITOR METHYLGLYOXAL BIS(GUANYLHYDRAZONE) BUT NOT BY ANALOGOUS METHYLGLYOXAL BIS(BUTYLAMIDINOHYDRAZONE)

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Abstract—The activities of ornithine decarboxylase (ODC) and spermidine/spermine N^1-acetyltransferase (SAT) were increased by the addition of *S*-adenosylmethionine decarboxylase (AdoMetDC) inhibitor methylglyoxal bis(guanylhydrazone) (MGBG) in cultured human erythroid leukemia K 562 cells. ODC activity began to increase 4 hr after the addition of the drug and attained a maximum at 12 hr. The increase of SAT activity lagged behind that of ODC activity. The increases of both ODC and SAT activities produced by MGBG were blocked by treatment with cycloheximide, suggesting that the increase of enzyme activity resulted from the synthesis of new enzyme proteins. The putrescine content in cells treated with MGBG increased markedly, whereas the levels of spermidine and spermine were depressed lower. On the other hand, methylglyoxal bis(butylamidinohydrazone) (MGBB), a derivative of MGBG inhibiting AdoMetDC effectively, did not induce ODC or SAT activities.

Methylglyoxal bis(guanylhydrazone) (MGBG)* is a known potent inhibitor of AdoMetDC, which is a key enzyme in the biosyntheses of the polyamines, spermidine and spermine [1, 2]. MGBG is also known as an antileukemic agent [3], and it has been suggested that the compound may accomplish its antineoplastic effects by virtue of the interference with polyamine metabolism. However, this turned out not to be the case since MGBG would accumulate within the cells to an extremely high level showing severe side effects [4–6]. The antiproliferative effect produced by MGBG correlates much better with the intracellular MGBG concentrations than with the depletion of polyamines [7, 8]. Furthermore, the inhibitory effects of MGBG towards polyamine biosynthesis in animals are transient because of the dramatically increased AdoMetDC protein that is stabilized by MGBG [9, 10]. Nevertheless, putrescine levels in MGBG-treated animals are markedly increased and there is some reduction in spermidine concentration [11–13].

Conversion of spermidine into putrescine occurs in mammalian cells by the combined action of two enzymes, SAT and polyamine oxidase [14–16]. N^1-Acetylspermidine, which is formed by the reaction of the former enzyme, is rapidly degraded by polyamine oxidase yielding putrescine and acetylaminopropanol [15, 16]. Polyamine oxidase is present in most tissues in great excess, and the degradation of spermidine is considered to be controlled by the activity of SAT which is highly inducible [16–19]. Karvonen and Pösö [20] reported that spermidine acetylase was enhanced after MGBG treatment in rat liver extracts. Pegg *et al.* [19] also noted a very high level of SAT in liver extracts from a rat treated with MGBG.

In this paper, we describe that ODC and SAT activities were induced by MGBG with different time-courses in human erythroid leukemia K 562 cells. These effects of MGBG are not observed with analogous methylglyoxal bis(butylamidinohydrazone) (MGBB). The latter compound has been shown to inhibit ODC and spermidine synthase in addition to AdoMetDC while the former compound inhibited only AdoMetDC [21].

MATERIALS AND METHODS

Chemicals. MGBG dihydrochloride was purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. MGBB was synthesized as described elsewhere [22], principally according to the method previously published [23]. DL-[1-^{14}C]Ornithine (sp. act. 48.8 mCi/mmol) and [acetyl-1-^{14}C]acetyl-CoA (sp. act. 48.8 mCi/mmol) were obtained from New England Nuclear, Boston, MA, U.S.A. All other chemicals were products of Nakarai Chemicals Ltd, Japan.

Cell culture. Human erythroid leukemia K 562 cells were grown at 37° in RPMI 1640 medium (Gibco, Uxbridge, U.K.), supplemented with 10% fetal calf serum, penicillin (50 i.u./ml) and streptomycin (50 μg/ml) in a 95% air–5% CO_2 humidified

* Abbreviations: MGBG, methylglyoxal bis(guanylhydrazone); MGBB, methylglyoxal bis(butylamidinohydrazone); ODC, ornithine decarboxylase; SAT, spermidine/spermine N^1-acetyltransferase; and AdoMetDC, *S*-adenosylmethionine decarboxylase.

incubator. Stock solutions of MGBG and MGBB were made in RPMI 1640 medium. The stock solutions were stored at 4°.

Preparation of enzyme extracts. Human erythroid leukemia K 562 cells (5×10^5) were seeded in 5-ml culture flasks. The cells were harvested at the indicated times, washed twice in 10 ml phosphate-buffered saline and centrifuged (1000 *g* for 3 min). The cell pellets (*ca* 2 μl) were suspended in 100 μl of 50 mM Tris–HCl buffer (pH 7.2) containing 10 mM dithiothreitol, 0.1 mM EDTA and 50 μM pyridoxal-5′-phosphate. The cell suspension was frozen and thawed three times, centrifuged at 12,000 *g* for 5 min and the supernatant was taken for ODC and SAT assays.

Assay for ODC. ODC activity was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine as described by Seely *et al.* [24]. The assay mixture contained 0.1 mM DL-[1-^{14}C]ornithine (0.2 μCi), 20 μM pyridoxal-5′-phosphate, 5 mM dithiothreitol, 50 mM Tris–HCl, pH 7.2, and enzyme extract in a final volume of 0.2 ml.

Assay for SAT. SAT activity was determined by measuring the incorporation of the acetyl-1-^{14}C group of acetyl-CoA into acetylspermidine as described by Matsui and Pegg [25]. The assay mixture contained 3 mM spermidine, 8 μM [acetyl-1-^{14}C]acetyl-CoA, 50 mM Tris–HCl (pH 7.8) and enzyme extract in a final volume of 0.2 ml.

Determination of polyamines. The cells were harvested by low speed centrifugation (1000 *g* for 3 min), washed twice in phosphate-buffered saline, suspended in 0.4 N perchloric acid and disintegrated by freeze-thawing three times. After centrifugation for 30 min at 10,000 *g*, the resulting supernatant fractions were used for polyamine determination by HPLC (Shimazu IC-5A) as described previously [22].

RESULTS

Induction of ODC by MGBG in K 562 cells

When K 562 cells were exposed to MGBG, there was a large increase in the activity of catalysis of the decarboxylation of ornithine (Fig. 1). This activity

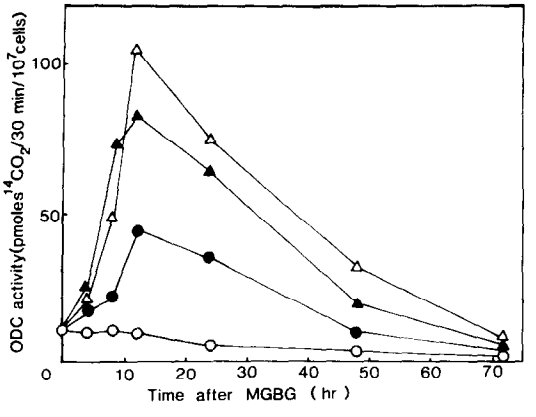

Fig. 1. Time-course of induction of ODC activity by MGBG. K 562 cells were incubated in the absence of (○) or presence of 1 μM (●), 2.5 μM (△) or 5.0 μM (▲) MGBG and at the times shown ODC activity was measured. Each point is the mean of duplicate experiments.

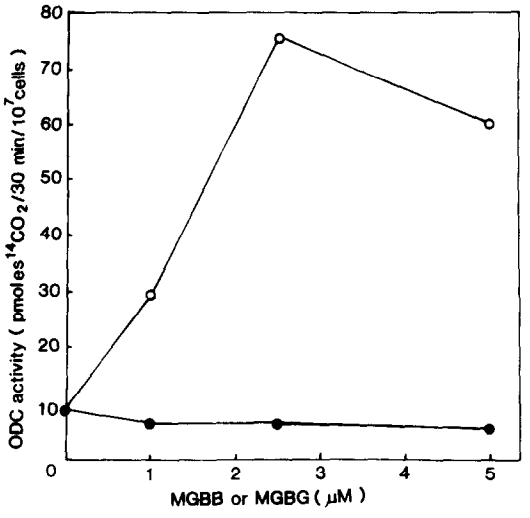

Fig. 2. Dose-dependent induction of ODC activity by MGBG (○) or MGBB (●). K 562 cells were exposed to the various concentrations of MGBG or MGBB for 12 hr before cell extracts were prepared to assay the ODC activity. Each point is the mean of duplicate experiments.

of ODC was elevated by 5-, 10- and 8-fold 12 hr after the addition of 1, 2.5, and 5 μM MGBG, respectively. The increased activities were then diminished to the basal level by 72 hr.

The effect of MGBG or MGBB concentration on the induction of ODC activity is shown in Fig. 2. The maximal ODC activity was obtained with 2.5 μM MGBG. Unlike MGBG, MGBB did not induce ODC activity at any concentration tested. This increase by MGBG of ODC activity was completely abolished by the presence of cycloheximide in the culture medium (Table 1).

The half-life of ODC was determined in K 562 cells by adding cycloheximide 12 hr after MGBG. As shown in Fig. 3, ODC activity in K562 cells

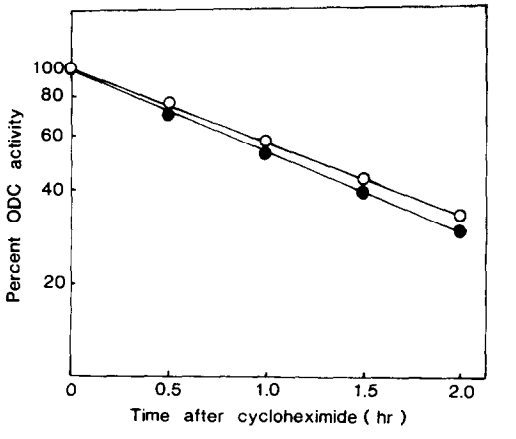

Fig. 3. Half-life of ODC activity in K 562 cells. The activity was measured in K 562 cells treated with 200 μM cycloheximide to prevent protein synthesis. The initial time point corresponds to the time of addition of cycloheximide. Results are expressed as the percentage of this value present at the time shown in K 562 cells treated with 2.5 μM MGBG (●) or untreated (○) for 12 hr. Each point is the mean of duplicate experiments.

Table 1. Effect of cycloheximide on induction of ODC by MGBG in K 562 cells

Treatment	ODC activity (pmol/30 min/10^7 cells)	(%)
None	9.6	100
MGBG (2.5 μM)	125.3	1305
MGBG (2.5 μM) + cycloheximide (50 μM)	9.2	95

K 562 cells were exposed to 2.5 μM MGBG and 50 μM cycloheximide and 8 hr later harvested to determine the ODC activity. Each value is the mean of duplicate experiments.

treated with MGBG declined in a similar manner to that of untreated cells in which protein synthesis was also blocked with cycloheximide.

The *in vitro* effect of MGBG or MGBB on ODC activity in the cell extracts was examined. The addition of MGBG to the reaction mixture at concentrations up to 0.1 mM did not show any stimulatory or inhibitory effect on ODC activity. However, unlike MGBG, MGBB inhibited ODC activity by 95% at a concentration of 0.1 mM as has been described elsewhere [21].

Induction of SAT activity by MGBG in K 562 cells

The effects of MGBG or MGBB on SAT activity in K 562 cells are shown in Fig. 4. In contrast to ODC, SAT activity was increased with longer time course after the addition of MGBG. The increase of SAT activity was about 23-fold 72 hr after the addition of 5 μM MGBG. MGBB, on the other hand, did not induce SAT activity at all. Experiments using other concentration (2.5 and 10 μM) of MGBB also showed no induction of SAT activity.

As shown in Fig. 5, the increase of SAT activity was dependent on the dose of MGBG. With 5 μM MGBG, SAT was enhanced by more than 24-fold. The results of experiments with higher concentrations of MGBG than 5 μM were not analyzable because of its toxic effect on K 562 cells. The increase of SAT activity produced by MGBG was diminished by the treatment of cycloheximide (Table 2).

The half-life of SAT activity was also determined in K 562 cells using cycloheximide after 72 hr of MGBG induction. As shown in Fig. 6, SAT activity in the cells treated with MGBG declined with a half-life of 80 min after cycloheximide addition. The half-life of SAT activity in untreated cells could not be reliably estimated because of its low basal value.

Figure 7 shows the effect of MGBG on the stability of SAT in the extracts from K 562 cells. SAT activity of the cell extracts in the presence of 5 μM MGBG declined at the same decay rate as that in the absence of the drug.

The *in vitro* effects of MGBG or MGBB on SAT activity in K 562 cells extracts were also examined. At lower concentrations, these compounds showed negligible effects on the enzyme activity. However, 0.1 mM MGBG or MGBB inhibited SAT activity by 40 and 29%, respectively. MGBG and MGBB were thus found to be rather weak inhibitors of SAT.

Effect of MGBG on polyamine synthesis and growth of K 562 cells

The effects of concentrations of MGBG on cellular polyamine contents and growth of K 562 cells are shown in Table 3. The drug decreased the intracellular contents of both spermidine and spermine dose-dependently and the growth of the cells was

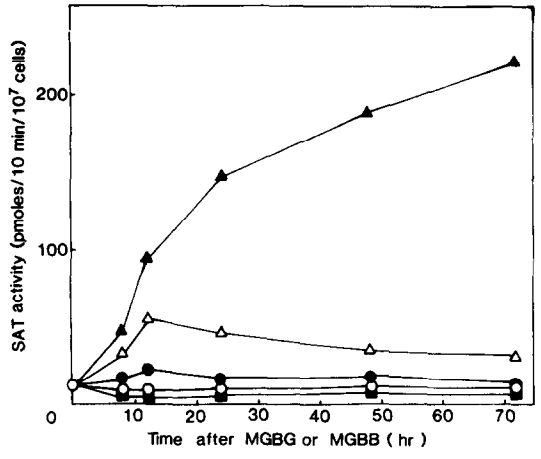

Fig. 4. Time-course of induction of SAT activity by MGBG or MGBB. K 562 cells were incubated in the absence (○) or presence of 1 μM (●), 2.5 μM (△) or 5.0 μM (▲) MGBG and in the presence of 5 μM MGBB (■). SAT activity was measured at the times shown. Each point is the mean of duplicate experiments.

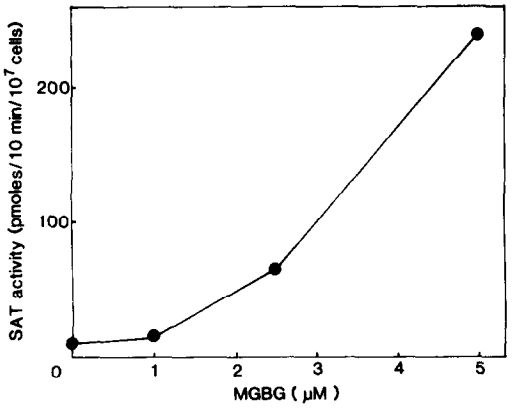

Fig. 5. Dose-dependence of SAT induction by MGBG. K 562 cells were exposed to the various concentrations of MGBG, and 72 hr later cell extracts were prepared and assayed for SAT activity. Each point is the mean of duplicate experiments.

Table 2. Effect of cycloheximide on induction of SAT by MGBG in K 562 cells

Treatment	SAT activity (pmol/10 min/10^7 cells)	(%)
None	9.7	100
MGBG (5 μM)	236.6	2439
MGBG (5 μM) + cycloheximide (50 μM)	99.4	1024
MGBG (5 μM) + cycloheximide (100 μM)	64.1	661
MGBG (5 μM) + cycloheximide (200 μM)	47.5	489

K 562 cells were exposed to 5 μM MGBG for 72 hr before addition of various concentrations of cycloheximide (50–200 μM). After 3 hr of incubation with cycloheximide, cell extracts were prepared and assayed for SAT activity. Each value is the mean of duplicate experiments.

Table 3. Effect of MGBG on polyamine contents and growth of K 562 cells

MGBG (μM)	Putrescine	Spermidine (amol/cell)	Spermine	Cell density ($\times 10^6$/flask)
0	243	2667	1867	5.90
2.5	5723	1702	954	5.45
5.0	3679	1182	766	4.50
10.0	1147	984	744	3.05

K 562 cells were exposed to increasing concentrations of MGBG for 72 hr. Experimental details for polyamine determinations are described in Materials and Methods. Each value is the mean of duplicate experiments.

concomitantly inhibited. To the contrary, large increases (5- to 25-fold) in putrescine content were observed in the cells exposed to MGBG.

DISCUSSION

The present study showed that the AdoMetDC inhibitor MGBG increased the activities of ODC and SAT by enhancing the synthesis of new enzyme proteins. The finding that MGBG, but not MGBB, induces ODC and SAT activities suggests that the guanyl group of MGBG is necessary to induce ODC and SAT activities. The results obtained with MGBB suggested that the inductions of ODC and SAT were not directed by the depletion of polyamines. We reported previously that MGBB depressed the intracellular levels of putrescine, spermidine and spermine simultaneously in K 562 cells resulting in strong inhibition of cell growth [22]. Decrease in putrescine concentration in MGBB-treated cells is also noteworthy because putrescine is increased in MGBG-treated cells as was seen in Table 3.

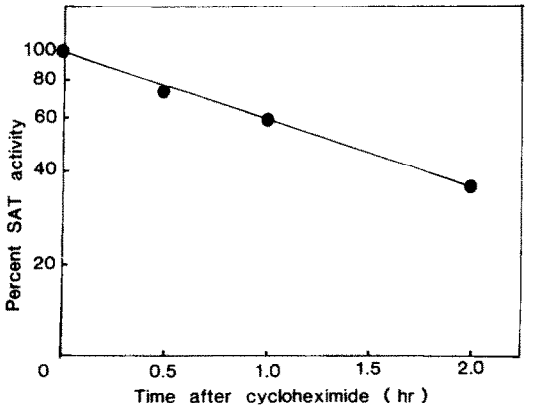

Fig. 6. Half-life of SAT activity in K 562 cells. The activity was measured in the cells treated with 200 μM cycloheximide to prevent protein synthesis. Results are expressed as the percentage of this value present at the times shown in K 562 cells treated with 5 μM MGBG for 72 hr. Each point is the mean of duplicate experiments.

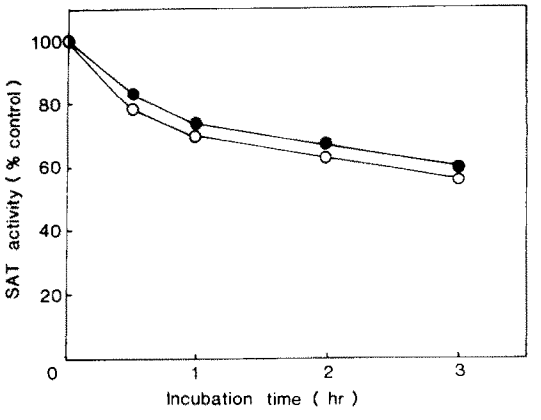

Fig. 7. Effect of MGBG on degradation of SAT in the cell extracts from K 562 cells. The cells were exposed to 5 μM MGBG, 72 hr later cell extracts were prepared and dialyzed overnight to remove MGBG. The dialyzed cell extracts were incubated at 37° in 25 mM sodium phosphate (pH 7.2) containing 0.3 mM EDTA and 0.5 mM DTT in the absence (○) or presence (●) of 5 μM MGBG. At the times shown, SAT activity was measured diluting by 50 times in the assay mixture. Each point is the mean of duplicate experiments.

Exposure of cells to MGBG brings about a number of effects on polyamine metabolism which could increase the levels of putrescine. These are: (a) an increase in ODC activity [26, 27]; (b) inhibition of diamine oxidase [28, 29]; (c) inhibition of AdoMetDC activity which indirectly increases putrescine by reducing the supply of carboxylated AdoMet for spermidine synthase [1, 2]; and (d) the induction of SAT connected to polyamine oxidase pathway which converts spermidine and spermine into putrescine. It is therefore not surprising that very large increases in putrescine have been reported to occur in cells of animals treated with MGBG [11–13] since these four distinct mechanisms act to increase putrescine.

The exact contribution of each of these mechanisms is likely to depend on the dose and time of administration. Thus, MGBG was shown to affect the cellular polyamine metabolism not only by inhibiting AdoMetDC and diamine oxidase but also by the inductions of ODC and SAT.

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