PARADOXICAL ENHANCEMENT OF S-ADENOSYLMETHIONINE DECARBOXYLASE IN RAT TISSUES FOLLOWING ADMINISTRATION OF THE SPECIFIC INHIBITOR METHYL GLYOXAL BIS(GUANYLHYDRAZONE)

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Abstract: Treatment of rats with large but sublethal doses of methyl glyoxal bis(guanylhydrazone), a potent in vitro inhibitor of animal S-adenosylmethionine decarboxylases, causes marked increases in the enzyme activity of extracts of kidney, ventral prostate, and testis which had been extensively dialyzed to remove any remaining drug. One day after administration of the inhibitor to female rats, the renal S-adenosylmethionine decarboxylase activity was 12 times the normal level and remained greatly enhanced for a further 24 hr. As indicated by decline in decarboxylase activity following depression of protein biosynthesis by injection of cycloheximide, the apparent half-life of the kidney enzyme in normal female rats is roughly 2 hr; in contrast, the apparent half-life of the enzyme is elevated to a value of more than 20 hr in animals that were previously treated with methyl glyoxal bis(guanylhydrazone). The increased renal S-adenosylmethionine decarboxylase activity following administration of the specific enzyme inhibitor in vivo may thus be due, at least in part, to stabilization of the enzyme against intracellular inactivation as a result either of direct combination of the enzyme protein with the inhibitor, or with substance(s) in the tissue whose levels are influenced by treatment with methyl glyoxal bis(guanylhydrazone).

Methyl glyoxal bis(guanylhydrazone) (MGBG) is an anti-leukemic agent whose antiproliferative actions against certain normal and malignant tissues can be prevented by administration of spermidine (1). MGBG is a very potent inhibitor of putrescine-activated S-adenosylmethionine decarboxylases of mammalian tissues and yeast, but depresses the action of the Mg^{++}-activated and putrescine-insensitive E. coli enzyme only at very much higher concentrations (2). MGBG does not inhibit L-ornithine decarboxylase and spermidine synthase, the two other enzymes besides S-adenosylmethionine decarboxylase that are responsible for spermidine synthesis in animal cells (3). The sensitivity of animal tissue S-adenosylmethionine decarboxylases to MGBG is much less when the activity is measured in the absence as compared with the presence of amine activators such as putrescine (4).

Administration of MGBG to rats (5), or exposure of plant lectin-stimulated lymphocytes to the drug (6,7,8) prevents the incorporation of labeled putrescine into spermidine,

and leads to an increased accumulation of the polyamine precursor putrescine in cells. In intact animals, however, inhibition of renal spermidine and spermine formation following injection of MGBG was found to be transitory; depression of the incorporation of labeled putrescine into spermidine and spermine occurred for only 8 to 10 hr after injection of MGBG, whereas by 20 hr after giving the drug, polyamine synthesis from labeled putrescine was actually greater than in control rat kidneys (5). This communication describes studies on the activity of S-adenosylmethionine decarboxylase in dialized tissue extracts after treatment of rats with MGBG. Paradoxical increases in the enzyme that were found to occur in a number of tissues may provide a possible explanation for the previously reported transitory nature of inhibition of spermidine and spermine biosynthesis by MGBG in vivo. The rapid decline in kidney S-adenosylmethionine decarboxylase due to administration of the protein synthesis inhibitor cycloheximide to normal rats is greatly diminished in animals that were treated with MGBG, suggesting that administration of the drug in vivo somehow stabilizes the enzyme against intracellular degradation.

EXPERIMENTAL PROCEDURES

The dihydrochloride salt of MGBG and cycloheximide were dissolved in 0.9% NaCl and administered to rats by intraperitoneal injection. Female rats of the Wistar strain (150 g body weight) and male rats of the Sprague-Dawley strain (350 g body weight) were employed. The animals were provided with rat cake and water ad libitum. Organs were removed from the animals immediately after death, and were homogenized at 2° with a medium containing either 10 mM Tris-HCl, 5 mM dithiothreitol, 0.1 mM EDTA of pH 7.5 at 4° (female rats) or 10 mM sodium phosphate, 2 mM dithiothreitol, 0.1 mM EDTA of pH 7.2 (male rats). The homogenates were centrifuged at 30,000 x g for 1 hr and the supernatant fluid was dialyzed for 12 hr against large volumes of 10 mM Tris-HCl, 10 mM 2-mercaptoethanol (female rats) or against the homogenization medium (male rats). S-adenosylmethionine decarboxylase was estimated as previously described (9) in a medium containing 100 mM sodium phosphate of pH 7.2; 0.2 mM S-adenosylmethionine-^{14}COOH; 2.5 mM putrescine; 5 mM dithiothreitol and suitable amounts of tissue extract. The decarboxylase activities were proportional to the amount of tissue extracts added.

RESULTS AND DISCUSSION

When the S-adenosylmethionine decarboxylase activities of crude centrifuged extracts of various rat tissues were measured in the presence of saturating levels of the activator putrescine at different times after administration of large but sublethal doses of MGBG

(180 to 295 μmoles per kg), the values obtained within the first 24 hr after giving the drug were extremely variable, and often abnormally low. This can doubtless be attributed to the presence of variable amounts of MGBG remaining in the fresh tissue extracts used for the enzyme assay, since the concentrations of MGBG required for 50% inhibition of the decarboxylase in vitro were in the range of 0.3 to 1 μM with the various tissues examined. If the centrifuged tissue extracts were first dialyzed against buffered thiol-containing solutions, however, then it was unexpectedly found that there was a highly significant enhancement of S-adenosylmethionine decarboxylase activities within a few hr after injection of MGBG (the dialysis procedures used had very little effect on the enzyme activities of control centrifuged tissue extracts). Thus, in the typical experiment shown in Table 1, the

Table 1. EFFECT OF ADMINISTRATION OF METHYL GLYOXAL BIS(GUANYLHYDRAZONE) ON ADENOSYLMETHIONINE DECARBOXYLASE ACTIVITIES OF DIALYZED KIDNEY EXTRACTS

Time after injection of MGBG (Hr)	Renal adenosylmethionine decarboxylase activity (pmoles CO_2/mg protein/30 min)
0	98 ± 36
3	204 ± 37
8	424 ± 81
27	1242 ± 353
48	1206 ± 363
96	177 ± 14
168	111 ± 29

Female rats were given MGBG (294 μmoles per kg) at the times before death indicated. The values given are the mean ± standard error for groups of 4 rats in each instance.

renal decarboxylase activity of female rats attained a peak value of nearly 12 times that of control kidney extracts by 27 hr after injection of a single dose of MGBG (294 μmoles per kg); the decarboxylase activity declined rather slowly thereafter and did not return to within the normal range until 168 hr after giving the drug.

More prolonged treatment with MGBG also resulted in a marked elevation of S-adenosylmethionine decarboxylase of a number of different rat tissues. In a representative experiment with male rats, for example, daily injections of MGBG (172 μmoles per kg) for 3 days increased the enzyme activities of dialyzed extracts of kidney by 5-fold, of ventral prostate by 6-fold, and of testis by 4-foid.

The paradoxical increase in S-adenosylmethionine decarboxylase activity of various tissues after in vivo treatment with MGBG, which directly inhibits the enzyme in vitro, could obviously be determined by many factors. These could conceivably include some effects of the drug on the levels or activity of endogenous activators or inhibitors of the decarboxylase present in tissues, or to some influence of the drug or its metabolites on the complex intracellular factors that control the balance between the rates of synthesis and breakdown (or inactivation) of the enzyme protein. The latter eventuality was examined by measuring the decrease in S-adenosylmethionine decarboxylase after virtual complete inhibition of cytoplasmic non-mitochondrial protein synthesis evoked by administration of cycloheximide, which does not directly affect S-adenosylmethionine decarboxylase even at high concentrations. Control female rats, as well as animals that were given MGBG 19 hr previously, were injected intraperitoneally with amounts of cycloheximide known to cause rapid and almost complete cessation of protein biosynthesis in many rat tissues (10); the animals were killed at various intervals after giving cycloheximide, extracts of kidney were prepared and dialyzed, and their S-adenosylmethionine decarboxylase activity was determined. The results shown in Figure 1 indicate that there was a rapid fall of renal decarboxylase activity of control kidney extracts, which reached 50% of the normal value at a time of slightly less than 2 hr after injection of cycloheximide (the literature reports similarly obtained estimates of the apparent half-life of S-adenosylmethionine decarboxylase of 35 min for regenerating liver (11) and of 60 min for uterus (12) in rats). In the animals that had been previously treated with MGBG, however, the decline in renal decarboxylase activity following cycloheximide treatment was strikingly decreased. Assuming that cycloheximide injection, and the attendant depression of protein biosynthesis, do not radically alter the normal rate of intracellular degradation of the enzyme, it can be calculated from the data of Figure 1 that the apparent half-life of renal S-adenosylmethionine decarboxylase in rats that did not receive MGBG was less than 2 hr whereas the corresponding value for the MGBG-injected animals was greater than 21 hr. The latter value is only approximate(and perhaps an under-estimate) because the high toxicity of the doses of cycloheximide employed precluded enzyme activity measurements at periods of more than 5 hr after giving the protein synthesis inhibitor. Whether the decline of S-adenosylmethionine decarboxylase after administration of cycloheximide is a reflexion of the rate of degradation of the enzyme protein, and whether MGBG has any influence on the rate of synthesis of the decarboxylase, remain unknown. Nevertheless it is clear from the data of Figure 1 that the stability of renal S-adenosylmethionine decarboxylase in vivo appears to be

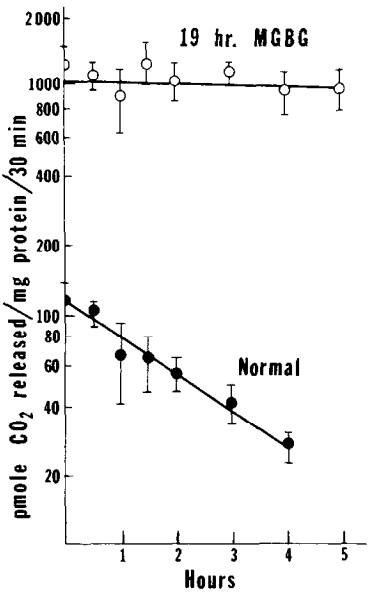

Fig. 1. Effect of cycloheximide administration on adenosylmethionine decarboxylase activity of dialyzed kidney extracts from normal and MGBG-treated female rats. Control animals received 0.5 ml of 0.9% NaCl intraperitoneally. The rats treated with MGBG received 290 micromoles of the drug per kg via the same route. After 19 hr, both sets of rats were given an intraperitoneal injection of cycloheximide (50 mg per kg) and groups of 4 control or MGBG-treated animals were killed at the time indicated. The mean values with bars indicating the standard error of the mean are plotted.

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increased by at least one order of magnitude by doses of MGBG that enhance the decarboxylase activity of dialyzed extracts to roughly the same degree.

It is well established that elevation of the activities of certain enzymes in animal tissues in vivo resulting from administration of substrates [e.g. tryptophan with respect to hepatic tryptophan pyrrolase (13)] or inhibitors [e.g. methotrexate with respect to the dihydrofolate reductase of certain malignant cells (14,15)] is due primarily to a decreased rate of degradation, rather than to an increased rate of synthesis, of the enzyme protein. It is conceivable that by combination with S-adenosylmethionine decarboxylase, the specific inhibitor MGBG could directly diminish the rate of intracellular breakdown of this enzyme. However, it is equally imaginable that a decreased degradation or inactivation of the decarboxylase could be due to some metabolite of MGBG, or that MGBG (or its metabolites) might influence the levels or activity of endogenous tissue constituents that in turn influence the activity of S-adenosylmethionine decarboxylase. In the latter connection, it has been previously reported (5) that MGBG administration causes significant increases in the steady-

state level of putrescine in kidney and other rat tissues. Although MGBG-induced alterations of intracellular putrescine concentrations cannot be completely ruled out as a factor leading to the enhanced stability of S-adenosylmethionine that occurs in the kidneys of MGBG-treated rats, it should be mentioned that the activities of the enzyme in dialyzed kidney extracts were not significantly affected either if the tissue was homogenized in the standard medium to which 2.5 mM putrescine was added, or as a result of administration of very large doses of putrescine (1136 μmoles per kg) daily for 3 days to normal male rats.

Filingame and Morris (7) have also found that S-adenosylmethionine decarboxylase activity is increased after cultures of stimulated lymphocytes are exposed to MGBG.

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