Biochemical Pharmacology, Vol. 45, No. 5, pp. 1174–1176, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

Stabilization of *S*-adenosylmethionine decarboxylase by aminoguanidine

(*Received* 7 *September* 1992; *accepted* 18 *December* 1992)

Abstract—Aminoguanidine, an inhibitor of copper-containing amine oxidases, is often used in cell culture studies to prevent the oxidation of added polyamines or their derivatives by the amine oxidase present in bovine serum. However, in the present study, we demonstrate that aminoguanidine, besides affecting the degradation of polyamines, may affect one of the important regulatory enzymes in the biosynthesis of polyamines, namely *S*-adenosylmethionine decarboxylase (AdoMetDC). Aminoguanidine was shown to induce a 2–3-fold increase in the AdoMetDC levels in L1210 cells. This increase was fully explained by a stabilization of the enzyme, probably caused by the binding of aminoguanidine to the active site of AdoMetDC.

The polyamines, putrescine, spermidine and spermine, which are present in all mammalian cells, play important roles in the regulation of cell growth and differentiation [1–3]. The degradation of polyamines in vertebrates is catalysed by two groups of enzymes; flavin-containing and copper-containing amine oxidases [4–6]. The polyamine oxidases are often capable of not only oxidizing polyamines, but also oxidizing various derivatives of the polyamines.

The presence of a copper-containing amine oxidase in bovine serum [5, 6], including foetal calf serum, has hampered the use of cell cultures in polyamine research. This problem can be overcome by the use of aminoguanidine, a potent inhibitor of various copper-containing amine oxidases, including diamine oxidase [7]. Thus, aminoguanidine (1 mM) is often added to the serum-containing growth medium when cells are supplemented with polyamines or their derivatives [8–12]. However, in spite of the frequent use of aminoguanidine and the fact that aminoguanidine in a strict chemical sense is a polyamine, there is very little information about the effects of aminoguanidine at other steps in polyamine metabolism. Williams-Ashman and Schenone [13] have reported that aminoguanidine at concentrations higher than 1 mM may inhibit one of the enzymes involved in polyamine synthesis, namely *S*-adenosylmethionine decarboxylase (AdoMetDC*) (EC 4.1.1.50). However, no numerical data was given. In the present study, we demonstrate that supplementing the growth medium with aminoguanidine may affect cellular AdoMetDC by inducing stabilization of the enzyme against degradation.

Materials and Methods

Cell culture. L1210 cells were grown in RPMI 1640 medium containing 10% foetal calf serum, 50 μM β-mercaptoethanol and antibiotics (50 IU of penicillin/mL and 50 μg streptomycin/mL). The cells were seeded at a density of 1.0×10^5 cells/mL in the presence or absence of 1 mM aminoguanidine. Cells were harvested 48 hr after seeding. When the turnover of AdoMetDC was determined, cycloheximide (50 μg/mL) was added for various times (30 min to 2 hr) before the cells were harvested.

Assays of AdoMetDC and ornithine decarboxylase activities. Extracts for measurements of AdoMetDC activity and AdoMetDC protein were prepared by sonicating the cells in 0.1 M Tris–HCl (pH 7.5) containing 0.1 mM EDTA and 0.5 mM dithiothreitol, followed by centrifugation at 20,000 *g* for 20 min (4°). AdoMetDC and ornithine decarboxylase activities were determined in aliquots of the supernatants by measuring the release of $^{14}CO_2$ from [carboxyl-^{14}C]*S*-adenosylmethionine and [1-^{14}C]ornithine, respectively, as described earlier [14, 15].

AdoMetDC radioimmunoassay. The amount of AdoMetDC protein was determined using a radioimmunoassay [14]. Aliquots of the 20,000 *g* supernatants were incubated at room temperature for 30 min with a monospecific antibody against rat prostate AdoMetDC, diluted 1:16,000. Pure rat AdoMetDC labelled with [*methyl*-^{3}H]AdoMet was then added and the samples were incubated for an additional 30 min. Antibody-bound radioactivity was determined after precipitation with bacterial protein A adsorbent (60 min) and centrifugation at 12,000 *g* for 2 min. Pure rat prostate AdoMetDC was used as standard.

Determination of polyamine content. Cellular polyamine content was determined as described previously [15] using an amino acid analyser (Biotronik LC 5001).

Results

In a study of the regulation of AdoMetDC in L1210 mouse leukemia cells we made the observation that the cellular content of AdoMetDC varied depending on the presence or absence of aminoguanidine in the growth medium. Thus, we carried out a more detailed study of the effects of aminoguanidine on this enzyme in the polyamine biosynthetic pathway. Cells were grown for 48 hr in the absence or presence of 1 mM aminoguanidine, whereupon the AdoMetDC activity as well as the AdoMetDC protein content was determined. The concentration used was similar to that used in most cell culture studies to block polyamine oxidation. As shown in Table 1, L1210 cells grown in the presence of aminoguanidine exhibited a 2–3-fold increase in AdoMetDC activity as well as AdoMetDC protein content. The activity of ornithine decarboxylase, on the other hand, was not affected by treatment with aminoguanidine (results not shown).

Figure 1 shows the effects of various concentrations of aminoguanidine on the activity of pure rat AdoMetDC. A small, but statistically significant decrease in the activity was seen when 1 mM aminoguanidine was added to the assay. However, a more dramatic effect was obtained with 10 mM aminoguanidine, which inhibited more than 75% of the AdoMetDC activity.

From the present results, it is clear that aminoguanidine, at high concentrations, binds to AdoMetDC, which consequently may affect the turnover of the enzyme. To determine whether the aminoguanidine-induced increase in AdoMetDC content was due to a stabilization of the enzyme we measured the half-life of AdoMetDC in cells grown in the absence or presence of aminoguanidine. As shown in Fig. 2, aminoguanidine treatment of the L1210

* Abbreviations: AdoMetDC, *S*-adenosylmethionine decarboxylase; MGBG, methylglyoxal bis(guanylhydrazone).

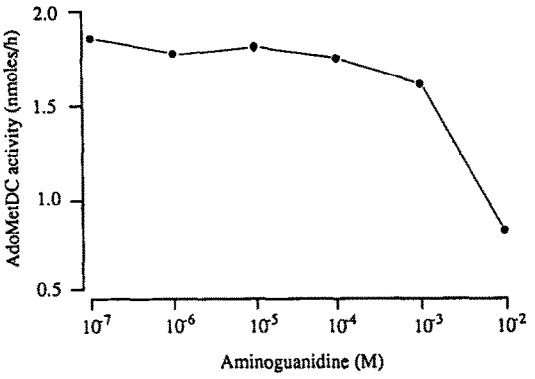

Fig. 1. Effects of aminoguanidine on the activity of pure rat AdoMetDC. Various concentrations of aminoguanidine were added to the assay of AdoMetDC activity. Mean ± SEM, N = 3. Error bars smaller than the symbols are not given.

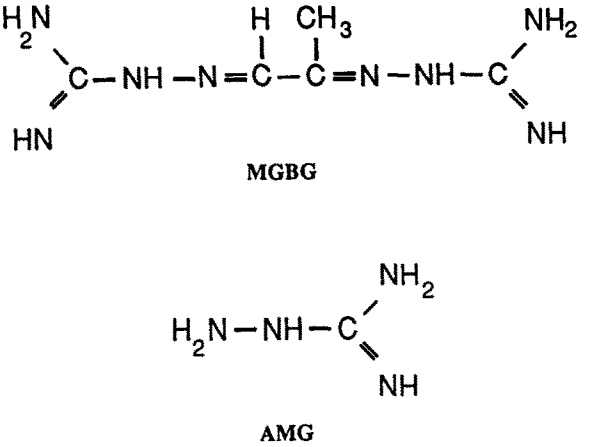

Fig. 3. Structural formulae of MGBG and aminoguanidine (AMG).

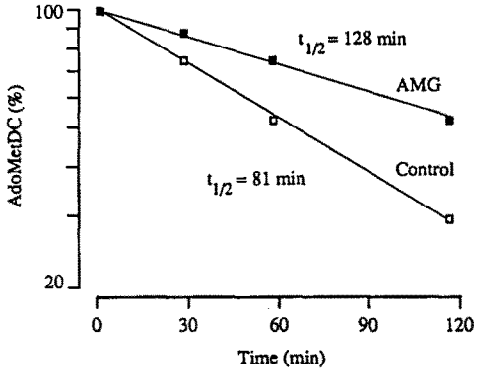

Fig. 2. Effects of aminoguanidine on the biological half-life of AdoMetDC in L1210 cells. Cells were grown in the absence (□) or presence (■) of 1 mM aminoguanidine for 48 hr. Cycloheximide (50 μg/mL) was added at various times before the cells were harvested. Error bars were within the symbols, N = 4.

cells gave rise to a stabilization of AdoMetDC. The increase in half-life caused by aminoguanidine was about 2-fold, which corresponded to the increase seen in the cellular AdoMetDC content.

Even though 1 mM aminoguanidine gave rise to a marked increase in the cellular content of AdoMetDC, there was no effect on the polyamine content in the L1210 cells (Table 1), which may indicate that the AdoMetDC activity in the L1210 cells was partly inhibited by treatment with aminoguanidine.

Discussion

Aminoguanidine is chemically related to methylglyoxal bis(guanylhydrazone) (MGBG), which contains two moieties of aminoguanidine (Fig. 3). MGBG is a potent inhibitor of AdoMetDC as well as of diamine oxidase [13, 16]. However, in spite of the fact that MGBG is an inhibitor of AdoMetDC, it gives rise to a paradoxical increase in the cellular levels of AdoMetDC [17, 18]. The MGBG-induced increase in AdoMetDC activity (measured in diluted extracts) has been demonstrated to be due to a stabilization of AdoMetDC [17, 18]. Thus, the binding of MGBG to the active site of AdoMetDC probably evokes a structural change in AdoMetDC, which renders the enzyme less susceptible to proteolytic degradation. Since the turnover of AdoMetDC is normally very fast, such a change rapidly gives rise to an increase in the cellular content of AdoMetDC. It is conceivable that aminoguanidine, although with less affinity than MGBG, can bind to AdoMetDC and cause a similar effect on the turnover of the enzyme.

In conclusion, our results demonstrate that aminoguanidine besides affecting the degradation of polyamines may affect one of the important regulatory steps in the biosynthesis of polyamines, namely that catalysed by AdoMetDC. This has to be taken into consideration whenever aminoguanidine is used in studies concerning polyamines.

Table 1. Effects of aminoguanidine on AdoMetDC and polyamine content in L1210 cells

	AdoMetDC		Polyamines‡		
Treatment	Activity*	Protein†	Putrescine	Spermidine	Spermine
Control	111 ± 5	1.52 ± 0.02	0.30 ± 0.04	2.89 ± 0.20	1.16 ± 0.11
Aminoguanidine	333 ± 5	2.96 ± 0.14	0.26 ± 0.04	2.69 ± 0.29	1.43 ± 0.18

Cells were grown for 48 hr in the absence or presence of 1 mM aminoguanidine.
*pmol/10^6 cells/hr; †ng/10^6 cells; ‡nmol/10^6 cells.
Values are means ± SD, N = 3–5.

Acknowledgements—This study was supported by grants from the Swedish Cancer Society, the Swedish Natural Science Research Council, the Medical Faculty, University of Lund, and the Magnus Bergvall, the Crafoord and the Torsten and Ragnar Söderberg foundations.

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