Effects of bis(guanylhya zones) on the activity and expression of ornithine decarboxylase

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Derivatives of glyoxal bis(guanylhya zone) (GBG), such as methylglyoxal bis(guanylhya zone) and ethylglyoxal bis(guanylhya zone), are potent inhibitors of *S*-adenosylmethionine decarboxylase (EC 4.1.1.50), the key enzyme required for the synthesis of spermidine and spermine. These compounds, but not the parent compound, induce a massive accumulation of putrescine, partly by blocking the conversion of putrescine into spermidine, but also by strikingly stimulating ornithine decarboxylase (ODC; EC 4.1.1.17) activity. The mechanism of the stimulation of ODC activity and enhanced accumulation of the enzyme protein apparently involved a distinct stabilization of the enzyme against intracellular degradation. However, although the parent compound GBG also stabilized ODC, it powerfully inhibited the enzyme activity and the accumulation of immunoreactive protein in cultured L1210 leukaemia cells. Kinetic considerations indicated that, in addition to the stabilization, all three compounds, GBG in particular, inhibited the expression of ODC. It is unlikely that the decreased rate of synthesis of ODC was attributable to almost unaltered amounts of mRNA in drug-treated cells, thus supporting the view that especially GBG apparently depressed the expression of ODC at some post-transcriptional level.

INTRODUCTION

Methylglyoxal bis(guanylhya zone) (MGBG), which is a powerful inhibitor of *S*-adenosylmethionine decarboxylase (Williams-Ashman & Schenone, 1972), also influences the metabolism of polyamines in several other ways. The drug inhibits diamine oxidase (EC 1.4.3.6) activity *in vitro* at micromolar concentrations (Hölttä *et al.*, 1973), almost totally abolishes intestinal diamine oxidase activity at doses required to inhibit spermidine and spermine formation *in vivo* (Kallio & Jänne, 1983), and stimulates ODC activity in tissues of experimental animals (Hölttä *et al.*, 1973; Heby *et al.*, 1973) as well as in cultured mouse tumour cells (Seppänen *et al.*, 1984a). The mechanism of the stimulation of ODC by the drug is not fully understood, yet it apparently involves a stabilization of the short-lived enzyme protein (Karvonen & Pösö, 1984). ODC is also stimulated in tumour cells exposed to ethylglyoxal bis(guanylhya zone) (EGBG), a close congener to MGBG (Seppänen *et al.*, 1984b). However, the parent compound, glyoxal bis(guanylhya zone) (GBG), although also powerfully inhibiting adenosylmethionine decarboxylase and hence spermidine synthesis, does not stimulate, but on the contrary inhibits, the activity of ODC (Seppänen *et al.*, 1984a).

We have now studied the effects of MGBG, EGBG and GBG on the activity and expression of ODC in cultured L1210 mouse leukaemia cells and reached the conclusion that not only is the stimulation of the enzyme activity by MGBG and EGBG fully explainable by enzyme stabilization, but, in addition, the bis(guanylhya zones), especially GBG, inhibit the expression of ODC, most likely at some post-transcriptional level.

EXPERIMENTAL

Cell cultures

Mouse L1210 leukaemia cells were cultured in RPMI 1640 medium supplemented with 5% (v/v) pooled human serum (Transfusion Service, Finnish Red Cross, Helsinki, Finland) and antibiotics (penicillin and streptomycin).

Chemicals

DL-[1-¹⁴C]Ornithine (sp. radioactivity 58 Ci/mol) was purchased from Amersham International (Amersham, Bucks., U.K.) and difluoro[³H]methionine (sp. radioactivity 26.5 Ci/mmol) from New England Nuclear (Boston, MA, U.S.A.). MGBG was obtained from Orion Pharmaceutical Company (Espoo, Finland). GBG was synthesized as described by Seppänen *et al.* (1984a). EGBG was generously given by Dr. Carl Porter (Roswell Park Memorial Institute, Buffalo, NY, U.S.A.).

Analytical methods

The activity of ODC was assayed as described by Jänne & Williams-Ashman (1971). One unit is defined as 1 pmol of CO₂ liberated in 30 min. Immunoreactive ODC was determined by the method of Erwin *et al.* (1983), by using monospecific antibody raised against mouse kidney ODC (Isomaa *et al.*, 1983). Total cellular RNA was isolated by the method of Auffray & Rougeon (1980). RNA was fractionated by electrophoresis in the presence of 6.7% (v/v) formaldehyde in 1.4% (w/v) agarose gel, transferred on to GeneScreen membrane (New England Nuclear Corp., Dreieich, West Germany) and hybridized to nick-translated (Rigby *et al.*, 1977) plasmid pODC16 (Jänne *et al.*, 1984) as described by Thomas (1980). RNA

Abbreviations used: GBG, glyoxal bis(guanylhya zone); MGBG, methylglyoxal bis(guanylhya zone) (mitogua zone); EGBG, ethylglyoxal bis(guanylhya zone); ODC, ornithine decarboxylase (EC 4.1.1.17).

Table 1. Effect of bis(guanylhyazones) on ornithine decarboxylase activity and the amount of immunoreactive protein in cultured L1210 cells

The cells were exposed to 1 μ M-MGBG or -GBG or to 5 μ M-EBG for 24 h.

Treatment	Ornithine decarboxylase		
	Activity (A) (units/mg of protein)	Protein (B) (pg/mg of protein)	A/B
None	695	1430	0.49
MGBG	1600	3080	0.52
GBG	72	566	0.13
EBG	2640	4070	0.65

dot-blot analyses were similarly performed by the method of Thomas (1980). Protein was determined by the method of Lowry *et al.* (1951).

RESULTS

An exposure of L1210 leukaemia cells to MGBG or EBG for 24 h resulted in a clear-cut stimulation of ODC activity, as shown in Table 1. The enhanced activity was accompanied by a similar increase in the amount of immunoreactive protein in cells exposed to MGBG or EBG (Table 1). In contrast with the alkylated derivatives, the parent compound GBG profoundly inhibited the enzyme activity, and to a lesser extent the accumulation of immunoreactive protein (Table 1). The discrepancy between enzyme activity and amount in GBG-exposed tumour cells indicates that in the presence of the drug large amounts of enzymically inactive protein accumulated. In any event, GBG does not inactivate ODC directly (Seppänen *et al.*, 1984a).

As shown in Fig. 1, a distinct prolongation of the half-life of ODC was apparently a major reason leading to the enhanced enzyme accumulation in tumour cells exposed to MGBG or EBG. Interestingly, GBG also appeared to stabilize the enzyme, yet not to the extent that MGBG and EBG did. The half-lives (min, $\pm$ S.E.M.) of ODC obtained from three separate experiments were as follows: untreated cells, 27.5 ± 8.3 ; GBG-exposed cells, 48 ± 17.7 ; MGBG-exposed cells, 78.5 ± 4.1 ; EBG-exposed cells, 81.3 ± 2.3 .

To elucidate the contribution of the lengthening of the half-life to the accumulation of ODC in bis-(guanylhyazone)-treated cells, we used the equation of Schimke & Doyle (1970), determining the relation of the rate of synthesis and degradation of a protein to the time-course needed to reach a new steady state of the protein accumulation. Using this equation and the experimental data of Table 1 together with the fact that $t_{1/2} = \ln 2 / K_d$, we obtained K_d , the first-order rate constant for degradation (min^{-1}), K_s , the zero-order rate constant for synthesis (expressed as enzyme units $\cdot \text{min}^{-1}$) and K_s'' , the zero-order rate constant for synthesis (expressed as enzyme amount $\cdot \text{min}^{-1}$). The values for these three rate constants are presented in Table 2. As shown, an exposure to any of the drugs resulted in an apparent inhibition of enzyme synthesis. This inhibition was most pronounced in cells exposed to GBG, as the apparent accumulation of ODC protein (K_s'') decreased to one-fifth of that found in untreated cells (Table 2). The marginal decrease in the rate constant for synthesis found in

Fig. 1. Effect of bis(guanylhyazones) on the half-life of ornithine decarboxylase in L1210 leukaemia cells

The tumour cells were exposed to 1 μ M-MGBG or -GBG or 5 μ M-EBG for 24 h, whereafter cycloheximide (10 μ g/ml) was added and the decay of the enzyme activity measured at the time points indicated. Half-lives: control cells (○) 20 min, GBG-treated cells (●) 38 min, MGBG-treated cells (□) 86 min, EBG-treated cells (■) 86 min.

EBG-treated cells may not be real, as this sort of kinetic approach is an approximation only.

We next measured the amounts of ODC mRNA with the aid of cloned cDNA encoding the enzyme. Fig. 2 depicts a Northern blot (Thomas, 1980) analysis in which RNA obtained from untreated and drug-treated leukaemia cells was fractionated by electrophoresis and probed with cDNA. As shown in Fig. 2, the amount of the major mRNA species (about 2.5 kilobases in size) was largely uninfluenced by the treatments. If anything, the amount of mRNA was higher in untreated leukaemia cells than in cells exposed to the drugs. A slight decrease in the mRNA amount in response to the drugs was confirmed by a separate experiment using dot-blot hybridization (results not shown).

It thus appears that, at least with GBG, the expression of ODC was blocked at some post-transcriptional level. Assuming that GBG, as a close congener of spermidine,

Table 2. Relative rates of degradation and synthesis of ornithine decarboxylase in bis(guanylhyazone)-treated L1210 leukaemia cells

Data were obtained from Table 1 and Fig. 1. K_d is the rate constant for degradation (min^{-1}); K'_s , the rate constant for synthesis (enzyme units $\cdot \text{min}^{-1}$); K''_s , the rate constant for synthesis (enzyme amount $\cdot \text{min}^{-1}$).

Treatment	Rate constant		
	K_d	K'_s	K''_s
None	0.0346	24.1	49.6
MGBG	0.0081	12.9	24.8
GBG	0.0182	1.3	10.3
EGBG	0.0081	21.4	32.8

Fig. 2. Ornithine decarboxylase mRNA amounts in untreated and bis(guanylhyazone)-exposed L1210 leukaemia cells

The tumour cells were treated with bis(guanylhyazones) as in Fig. 1; total RNA was extracted, fractionated (20 μg each) by electrophoresis, transferred on to hybridization membranes and hybridized to nick-translated pODC16. Lane 1, untreated cells; lane 2, MGBG-treated cells; lane 3, GBG-treated cells; lane 4, EGBG-treated cells. Abbreviation: kb, kilobases.

could possibly induce the formation of the macromolecular inhibitor 'antizyme' (Heller *et al.*, 1976), we tested GBG (at concentrations up to 10 μM) for possible antizyme induction. The results of these experiments gave no indication of an antizyme-based inhibition of ODC.

DISCUSSION

The present results show that the three bis(guanylhyazones) stabilize ODC, leading to an enhanced accumulation of the enzyme in MGBG- and EGBG-exposed cells, but not in GBG-treated cells. As the

accumulation of ODC mRNA appeared to be only slightly affected by the drug treatments, it is likely that the stimulation of ODC activity and accumulation are to large extent explainable by the stabilization of the enzyme protein. The apparent decrease in the rate of synthesis of ODC in cells exposed to MGBG may be attributable to a lower rate of general protein synthesis in response to the drug. However, the markedly decreased accumulation of ODC and the apparent decrease in the rate constant for enzyme synthesis produced by GBG is not explainable by a decrease in general protein synthesis, since this drug less effectively inhibits the synthesis of protein than does MGBG (Seppänen *et al.*, 1984a). As the accumulation of ODC mRNA appears to be largely unaffected by GBG treatment, and as 'antizyme' seems unlikely to be responsible, a post-transcriptional, most probably translational, inhibition remains the only explanation for the decreased accumulation of the enzyme protein in response to GBG.

The elucidation of the nucleotide sequence of ODC mRNA (Gupta & Coffino, 1985; Kahana & Nathans, 1985) has revealed that the mRNA of the enzyme contains an unusually long 5' non-coding segment of more than 700 (Kahana & Nathans, 1985) or more than 300 (Gupta & Coffino, 1985) nucleotides. This offers a possibility for translational control, as it is known that a long 5' untranslated region makes the translation inefficient, and deletion or shortening of this segment strikingly improves the translational efficiency (Thireos *et al.*, 1984; Darveau *et al.*, 1985).

It is thus possible that polyamines or their close analogues, such as GBG, could affect the secondary structure of the long 5' non-coding segment and modify (inhibit) the translation rate of ODC.

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REFERENCES

- Auffray, C. & Rougeon, F. (1980) *Eur. J. Biochem.* **107**, 303–314
- Darveau, A., Pelletier, J. & Sonenberg, H. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2315–2319
- Erwin, B. G., Seely, J. E. & Pegg, A. E. (1983) *Biochemistry* **22**, 3027–3032
- Gupta, M. & Coffino, P. (1985) *J. Biol. Chem.* **260**, 2941–2944
- Heby, O., Sauter, S. & Russell, D. H. (1975) *Biochem. J.* **136**, 1121–1124
- Heller, J. S., Fong, W. F. & Canellakis, E. S. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1858–1862
- Hölttä, E., Hannonen, P., Pispä, J. & Jänne, J. (1973) *Biochem. J.* **136**, 669–676
- Isomaa, V. V., Pajunen, A. E. I., Bardin, C. W. & Jänne, O. A. (1983) *J. Biol. Chem.* **258**, 6735–6740
- Jänne, J. & Williams-Ashman, H. G. (1971) *J. Biol. Chem.* **246**, 1725–1732
- Jänne, O. A., Kontula, K. K., Isomaa, V. V. & Bardin, C. W. (1984) *Ann. N.Y. Acad. Sci.* **438**, 72–84
- Kahana, C. & Nathans, D. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1673–1677
- Kallio, A. & Jänne, J. (1983) *Biochem. J.* **212**, 895–898
- Karvonen, E. & Pösö, H. (1984) *Biochim. Biophys. Acta* **791**, 239–243

- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251
- Schimke, R. T. & Doyle, D. (1970) *Annu. Rev. Biochem.* **39**, 929–976
- Seppänen, P., Fagerström, R., Alhonen-Hongisto, L., Elo, H., Lumme, P. & Jänne, J. (1984a) *Biochem. J.* **221**, 483–488
- Seppänen, P., Ruohola, H. & Jänne, J. (1984b) *Biochim. Biophys. Acta* **803**, 331–337
- Thireos, G., Penn, M. D. & Greer, H. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5096–5100
- Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201–5205
- Williams-Ashman, H. G. & Schenone, A. (1972) *Biochem. Biophys. Res. Commun.* **46**, 288–295

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