CGP 48664, a potent and specific *S*-adenosylmethionine decarboxylase inhibitor: effects on regulation and stability of the enzyme

Fredrik SVENSSON*, Helmut METT† and Lo PERSSON*‡

*Department of Physiology and Neuroscience, University of Lund, Sölvegatan 19, S-223 62 Lund, Sweden, and
†Pharmaceutical Division, Research Department, Novartis, K-125.4.01, Klybeckstrasse, CH-4002 Basel, Switzerland

Mammalian *S*-adenosylmethionine decarboxylase (AdoMetDC) catalyses a regulatory important step in the biosynthesis of polyamines and is a potential target for therapeutic agents against various parasitic diseases and proliferative disorders. In the present study we examined the effects of a newly synthesized AdoMetDC inhibitor, 4-amidinoindan-1-one 2′-amidinohydrazone (CGP 48664), on polyamine metabolism in the mouse leukaemia cell line L1210. Treatment of the cells with 2 μM CGP 48664 led to a depletion of cellular spermidine and spermine. The putrescine content, in contrast, was markedly increased. Cells seeded in the presence of the inhibitor showed a significant decrease in growth rate, which was fully reversed by the addition of 2 μM spermidine or 1 μM spermine. The syntheses of ornithine decarboxylase and AdoMetDC were greatly increased in cells treated with CGP 48664. These increases were not correlated with similar changes in the mRNA levels, indicating the involvement of a translational mechanism. CGP 48664 was demonstrated to be a very poor competitor of spermidine uptake in the L1210 cells. L1210 cells deficient in polyamine transport were as sensitive to the antiproliferative effect of the inhibitor as were the parental cells, indicating that CGP 48664 did not enter the cells by the polyamine transport system. In addition to inhibiting AdoMetDC, CGP 48664 stabilized the enzyme against degradation. In the present study we also demonstrated that aminoguanidine (AMG), which is frequently used in cellular systems to inhibit any action of serum polyamine oxidase, apparently inhibits AdoMetDC by an irreversible mechanism that markedly stabilizes the enzyme against proteolytic degradation. CGP 48664 and the parental compound methylglyoxal bis(guanylhydrazone), which is also a potent inhibitor of AdoMetDC, contain one or two AMG-like moieties; the importance of these residues in the inhibition of AdoMetDC is discussed.

INTRODUCTION

The polyamines putrescine, spermidine and spermine are essential for cell growth and differentiation, although the specific functions of these compounds still remain somewhat unclear [1–5]. The importance of the polyamines in cell growth makes the polyamine biosynthetic pathway a potential target for therapeutic agents against various parasitic diseases and proliferative disorders [4–7]. The regulatory steps in this pathway are catalysed by *S*-adenosylmethionine decarboxylase (AdoMetDC) and ornithine decarboxylase (ODC) [3,6,7]. The activities of these enzymes are normally very low in non-proliferating cells but increase as the cells enter the cell cycle in response to various growth stimuli. Inhibition of either of the enzymes results in a retardation of cell growth that can be reversed by the addition of polyamines.

ODC has generally been considered as the enzyme catalysing the rate-limiting step in polyamine biosynthesis. However, results from several studies indicate that in some systems the supply of decarboxylated *S*-adenosylmethionine is the actual rate-limiting factor in polyamine synthesis [2]. Mammalian AdoMetDC is regulated at a multitude of levels, including transcriptional, translational and post-translational [3,5,7]. The enzyme has a very rapid turnover, with a half-life of often less than 1 h. Hence any change in the synthesis or degradation of AdoMetDC will rapidly affect the cellular amount of the enzyme and thus the rate of polyamine synthesis. In mammalian cells AdoMetDC is synthesized as a proenzyme with a molecular mass of 38 400 Da, which is autocatalytically cleaved into two subunits of 30 700 and 7700 Da [8,9]. Both subunits have been demonstrated to be essential for the active enzyme. In this process a pyruvate group is generated, which acts as a prosthetic group of the active enzyme [10,11]. The conversion of the proenzyme into the active enzyme has been shown to be stimulated by putrescine both *in vitro* and *in vivo* [12–14]. In addition, putrescine has a direct stimulatory effect on the AdoMetDC activity [15].

Methylglyoxal bis(guanylhydrazone) (MGBG) is a frequently used inhibitor of AdoMetDC. MGBG is a polyamine analogue that enters the cells rapidly by the specific polyamine transport system [16]. It is a potent inhibitor of AdoMetDC, although with relatively low specificity. In addition to inhibiting AdoMetDC, MGBG stabilizes the enzyme against proteolytic degradation, inhibits diamine oxidase and induces the enzyme spermidine/spermine N^1-acetyltransferase, which catalyses the rate-limiting step in polyamine catabolism [7,16–19]. A common phenomenon observed after exposure of cells to MGBG is swelling of the mitochondria, with a subsequent decrease in ATP production, indicating other non-specific effects of this inhibitor [16]. More specific inhibitors of AdoMetDC have been prepared, which are analogues of *S*-adenosylmethionine or decarboxylated *S*-adenosylmethionine [20–23]. However, a major disadvantage of these inhibitors is their poor uptake into cells. Several MGBG analogues have been synthesized that were shown to be more potent and specific inhibitors of AdoMetDC than those used earlier [24]. In the present study we have examined the effects of

Abbreviations used: AdoMetDC, *S*-adenosylmethionine decarboxylase; AMG, aminoguanidine; MGBG, methylglyoxal bis(guanylhydrazone); ODC, ornithine decarboxylase.

‡ To whom correspondence should be addressed.

one of the most potent of these inhibitors, CGP 48664 [25], a cyclic analogue of MGBG, on polyamine metabolism in the mouse leukaemia cell line L1210.

EXPERIMENTAL

Materials

L-[1-^{14}C]Ornithine (54 mCi/mmol) and *S*-[*carboxy*-^{14}C]-adenosyl-L-methionine (58.9 mCi/mmol) were obtained from New England Nuclear (Du Pont Scandinavia, Stockholm, Sweden). L-[^{35}S]Methionine (1461 Ci/mmol) and [^{32}P]UTP (800 Ci/mmol) were purchased from Amersham International (Little Chalfont, Bucks., U.K.). Antibodies against mouse ODC and rat AdoMetDC were produced as described previously [26,27]. cDNA species encoding hamster ODC fragment [28] and human AdoMetDC [8] were kindly provided by Dr. I. E. Scheffler and Dr. A. E. Pegg respectively. The AdoMetDC inhibitor CGP 48664 was prepared as a dihydrochloride hydrate at Ciba-Geigy (Basel, Switzerland) [25].

Cell culture

L1210 cells were grown in RPMI 1640 medium containing 10% (v/v) foetal calf serum, 50 μM 2-mercaptoethanol and antibiotics (50 i.u./ml penicillin and 50 μg/ml streptomycin). Aminoguanidine (AMG; 1 mM) was added, except where stated otherwise, to inhibit any activity of polyamine oxidase present in bovine serum. In some experiments spermidine (2 μM) or spermine (1 μM) was added. Plateau-phase cells were seeded at a density of 10^5 cells/ml in the absence or presence of 2 μM CGP 48664 and harvested 1, 2, 3 or 4 days after seeding. The turnover of AdoMetDC was determined by measuring the decay in enzyme activity and protein after the addition of cycloheximide (50 μg/ml) to the medium.

Determination of ODC and AdoMetDC activities

Cells were sonicated in ice-cold 0.1 M Tris/HCl, pH 7.5, containing 0.1 mM EDTA and 2.5 mM dithiothreitol. After centrifugation for 20 min at 20000 ***g*** and 4 °C, ODC and AdoMetDC activities were determined in aliquots of the supernatants by measuring the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine and *S*-[*carboxy*-^{14}C]adenosyl-L-methionine respectively, as described previously [29].

AdoMetDC radioimmunoassay

Aliquots of the 20000 ***g*** supernatant of sonicated L1210 cells were incubated at room temperature with a specific antibody against rat prostate AdoMetDC diluted 1:16000 [27]. Purified rat enzyme labelled with *S*-[^{3}H]adenosylmethionine [27] was then added and the samples were incubated for an additional 30 min. Antibody-bound radioactivity was determined after precipitation with bacterial Protein A for 60 min and centrifugation at 12000 ***g*** for 2 min. Purified rat prostate AdoMetDC enzyme was used as standard [27].

Determination of rates of synthesis of ODC and AdoMetDC

The ODC and AdoMetDC synthesis rates were determined by measuring the incorporation of [^{35}S]methionine into the enzyme proteins. The cells were collected by centrifugation and reseeded in preheated (37 °C) methionine-free medium. After a 10 min preincubation at 37 °C, the cells were supplemented with [^{35}S]methionine (20 μCi/ml) and then incubated for an additional 20 min. The incorporation of radioactivity into proteins was stopped by the addition of 2 vol. of ice-cold medium containing 5 mM methionine. The cells were collected by centrifugation at 1000 ***g*** for 10 min at 4 °C and sonicated in 0.1 M Tris/HCl, pH 7.5, containing 0.1 mM EDTA, 2.5 mM dithiothreitol and 5 mM methionine. After centrifugation at 30000 ***g*** for 20 min at 4 °C, aliquots of the supernatants containing equal amounts of radioactivity were incubated with an excess of anti-AdoMetDC or anti-ODC antibody for 30 min at room temperature. The enzyme–antibody complex was precipitated by the addition of bacterial Protein A adsorbent (30 min at room temperature). The precipitate was washed four times with 10 mM Tris/HCl, pH 7.5, containing 0.1 mM EDTA, 2.5 mM dithiothreitol, 0.1% SDS, 0.1% Triton X-100 and 0.1% Tween-80. Precipitated ODC or AdoMetDC was fractionated by SDS/PAGE [12% (w/v) gel]. The radioactivity was detected by fluorography after the incubation of the gel in Amplify (Amersham). Rat AdoMetDC labelled with *S*-[^{3}H]adenosylmethionine, mouse ODC labelled with D,L-2-[3,4-^{3}H]difluoromethylornithine and [^{14}C]methylated proteins (Amersham) were used as molecular mass markers. Relative measurements of AdoMetDC and ODC syntheses were obtained by densitometric scanning.

Northern blot analysis

Total cellular RNA (20 μg) was isolated by the method of Chomczynski and Sacchi [30] and fractionated on a 1% (v/v) agarose gel in the presence of 2.2 M formaldehyde. The RNA was then transferred to a Hybond-N membrane (Amersham) and hybridized to ^{32}P-labelled ODC or AdoMetDC riboprobe. The membranes were washed at high stringency and analysed by fluorography. Relative measurements of ODC and AdoMetDC mRNA contents were obtained by densitometric scanning.

Determination of spermidine uptake

One day after seeding, cells were reseeded in preheated medium containing 2 μM [^{3}H]spermidine (0.5 Ci/mmol) and various concentrations of CGP 48664 (0–400 μM). After a 20 min incubation at 37 °C the uptake of labelled spermidine was stopped by the addition of an excess of non-radioactive spermidine and by placing the samples on ice. The cells were collected by centrifugation, washed once in PBS buffer containing 1 mM spermidine and then sonicated in 0.1 M Tris/HCl, pH 7.5, supplemented with 1 mM spermidine. The amount of radioactivity was determined by liquid-scintillation counting. The non-specific binding of labelled spermidine was measured at 0 °C.

Determination of polyamine content

The polyamine content was determined as described earlier [31] with an amino acid analyser (Biotronik LC 5001).

RESULTS

Reseeding plateau-phase L1210 cells in fresh medium resulted in a rapid growth of the cells, which was accompanied by an increase in both ODC and AdoMetDC activities (Figure 1). The enzyme activities were highest during the exponential growth period, giving peak values for ODC and AdoMetDC activities on day 1 or 2. Thereafter the enzyme activities declined as the cells were entering the plateau phase. Exposure of the cells to 2 μM CGP 48664 almost completely eradicated the growth-induced increase in AdoMetDC activity (Figure 1). In contrast, ODC activity increased even further in the presence of CGP 48664, reaching values 4–5-fold higher than those observed in the absence of the AdoMetDC inhibitor.

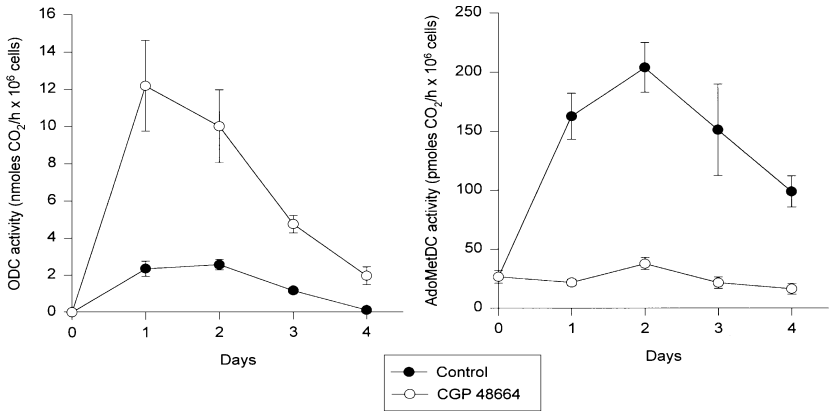

Figure 1 Effects of CGP 48664 on ODC and AdoMetDC activities in L1210 cells

Cells were seeded in the absence (●) or presence (○) of 2 μM CGP 48664. Cells were harvested daily and analysed for ODC and AdoMetDC activities. Each value is the mean ± S.E.M., $n = 3$ or 4.

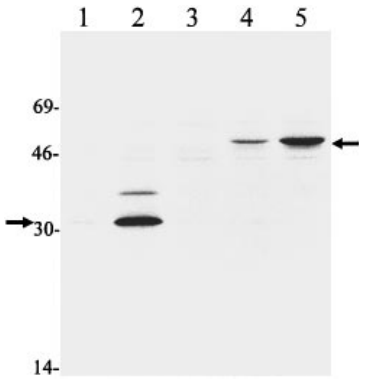

Figure 2 Effects of CGP 48664 on the synthesis of AdoMetDC and ODC in L1210 cells

Cells were grown for 1 (ODC) or 2 (AdoMetDC) days in the absence or presence of 2 μM CGP 48664 before being analysed for rate of AdoMetDC or ODC synthesis by pulse-labelling with [^{35}S]methionine. Lane 1, AdoMetDC, untreated control cells; lane 2, AdoMetDC, CGP 48664; lane 3, non-immune serum; lane 4, ODC, untreated control cells; lane 5, ODC, CGP 48664. The migrations of purified rat prostate AdoMetDC labelled with *S*-[^{3}H]adenosyl-L-methionine (molecular mass 31 kDa) and of purified mouse kidney ODC labelled with D,L-2-[3,4-^{3}H]difluoromethylornithine (molecular mass 53 kDa) are indicated. ^{14}C-labelled methylated proteins were used as molecular mass markers; positions of markers, in kDa, are shown at the left.

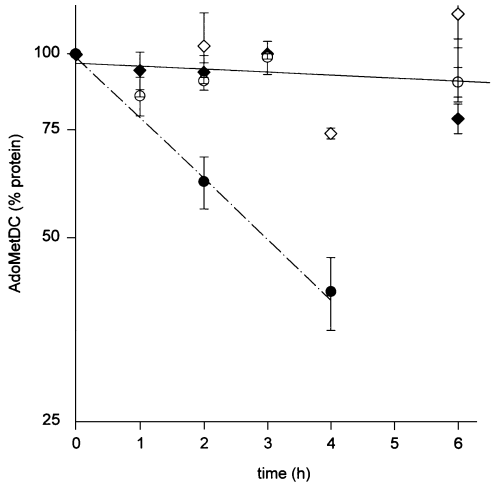

Figure 3 Effects of AMG and CGP 48664 on the turnover of AdoMetDC in L1210 cells

Cells were grown for 2 days in the absence or presence of 1 mM AMG and 2 μM CGP 48664. The turnover of AdoMetDC in the cells was determined by following the decay of AdoMetDC protein, using a radioimmunoassay, after the addition of cycloheximide (50 μg/ml). Symbols: ●, cells grown in the absence of AMG and CGP 48664; ◆, cells grown in the presence of AMG; ◇, cells grown in the presence of CGP 48664; ○, cells grown in the presence of AMG and CGP 48664. The solid line represents the mean turnover of AdoMetDC in cells grown in the presence of AMG and/or CGP 48664. Means ± S.E.M., $n = 3$.

The increase in ODC activity observed after treatment with CGP 48664 was almost fully explained by an increase in the rate of ODC synthesis, as measured by determining the incorporation of labelled methionine into the enzyme (Figure 2). In spite of the fact that almost no AdoMetDC activity was found in the cells seeded in the presence of the inhibitor, a more than 8-fold increase in the synthesis of AdoMetDC was observed in the cells 2 days after seeding. In addition to a major labelled protein with a molecular mass of approx. 31 kDa, corresponding well to that of the larger subunit of AdoMetDC [9], the AdoMetDC antibody precipitated a labelled protein with a molecular mass of approx. 38 kDa (Figure 2). The latter protein was most probably the proenzyme form of AdoMetDC. Most of the radioactivity was found in the 31 kDa protein, indicating a rapid conversion of the proenzyme form into its subunits.

Measuring the amount of AdoMetDC protein with a radioimmunoassay revealed an increase in cells exposed to the inhibitor (results not shown). To determine whether part of this increase was due to stabilization of the enzyme, we measured the turnover of the protein in cells seeded in the absence and in the presence of CGP 48664 (Figure 3). In a preliminary experiment we found that AdoMetDC protein was very stable in cells seeded in the absence of the inhibitor as well as in cells seeded in its presence. That AdoMetDC protein was stable in the cells grown in the absence of the inhibitor was of great surprise, because AdoMetDC usually has a very rapid turnover [7]. The cells were routinely grown in the presence of 1 mM AMG to inhibit any activity of serum polyamine oxidase. Thus it was conceivable

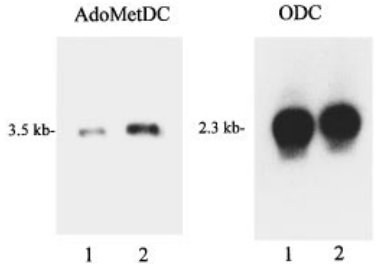

Figure 4 Effects of CGP 48664 on the levels of AdoMetDC mRNA and ODC mRNA in L1210 cells

The cells were seeded in the absence or presence of 2 μM CGP 48664 and then harvested for Northern blot analysis 1 (ODC mRNA) or 2 (AdoMetDC mRNA) days after seeding. Total cellular RNA was isolated and fractionated on a 1% (w/v) agarose gel containing formaldehyde, transferred to a Hybond-N membrane and hybridized with ^{32}P-labelled riboprobes for AdoMetDC or ODC. Lane 1, cells grown in the absence of CGP 48664; lane 2, cells grown in the presence of CGP 48664.

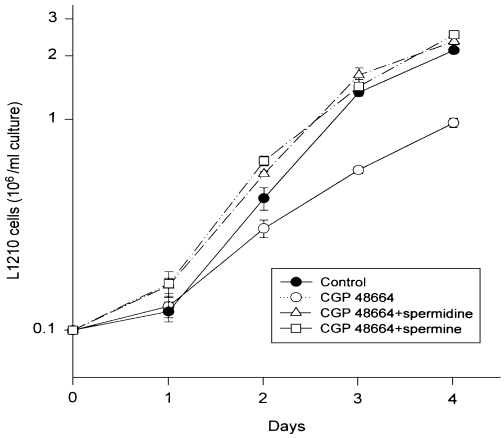

Figure 6 Effects of CGP 48664 on L1210 cell growth

Cells were seeded in the absence or presence of 2 μM CGP 48664 in combination with 2 μM spermidine or 1 μM spermine. Cells were counted daily. Symbols: ●, control cells; ○, cells grown in the presence of CGP 48664; △, cells grown in the presence of CGP 48664 and spermidine; □, cells grown in the presence of CGP 48664 and spermine. Each value is the mean ± S.E.M., $n = 3$.

that AMG stabilized the enzyme against degradation. A closer examination of the turnover of AdoMetDC protein in cells seeded in the absence or presence of AMG revealed that the half-life of the enzyme was between 2 and 3 h in cells grown in the absence of AMG, whereas the protein was almost stable ($t_{\frac{1}{2}}$ approx. 20 h) in cells grown in the presence of AMG, demonstrating a stabilizing effect of AMG on AdoMetDC (Figure 3). The half-life of AdoMetDC activity, in contrast, was much shorter than that of the AdoMetDC protein and was prolonged only slightly by AMG. In cells grown in the absence of AMG, the AdoMetDC activity decayed with a half-life of approx. 45 min after treatment with cycloheximide, whereas in cells grown in the presence of AMG the half-life of AdoMetDC activity was approx. 70 min (results not shown). Treatment with CGP 48664 also markedly stabilized the enzyme against degradation. The enzyme protein was apparently stable in the cells grown in the presence of CGP 48664 whether AMG was present or not (Figure 3).

Northern blot analysis of total RNA from the L1210 cells on day 1 after seeding revealed no difference in the amount of ODC mRNA in the cells exposed to CGP 48664 from that in the control cells (Figure 4). The amount of AdoMetDC mRNA, in contrast, was 2-fold higher on day 2 in the cells seeded in the presence of the inhibitor than in those seeded in the absence of the inhibitor (Figure 4).

The increase in ODC and AdoMetDC activities, occurring during exponential growth, was reflected in a transient increase in the cellular content of the polyamines putrescine, spermidine and spermine (Figure 5). Exposure of the cells to CGP 48664 eliminated the increase in spermidine and spermine content. In fact, a gradual depletion of these polyamines was observed in the cells treated with the AdoMetDC inhibitor (Figure 5). The putrescine content, in contrast, was greatly increased in cells seeded in the presence of the inhibitor, indicating an efficient block in the polyamine biosynthetic pathway between putrescine and spermidine (Figure 5).

Cells exposed to the AdoMetDC inhibitor exhibited a reduced growth, especially during the exponential growth period (days 1–3), when seeded in fresh medium (Figure 6). The growth

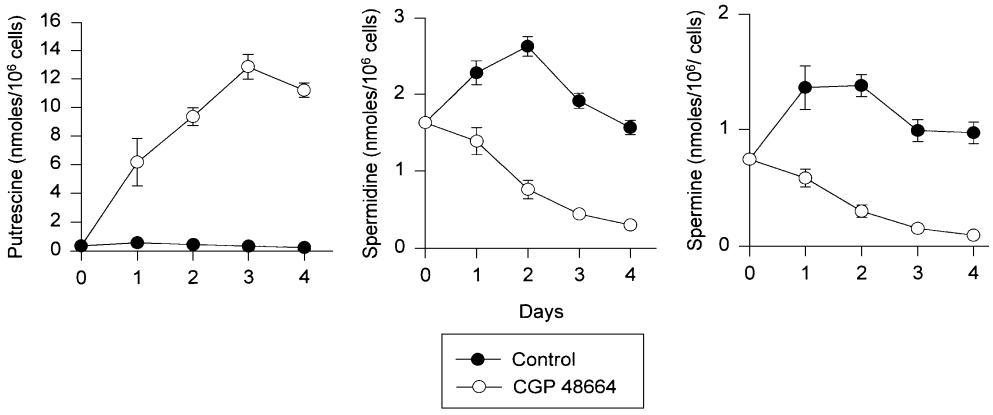

Figure 5 Effects of CGP 48664 on polyamine content in L1210 cells

Cells were seeded in the absence (●) or presence (○) of 2 μM CGP 48664. Cells were harvested daily and analysed for polyamine content. Each value is the mean ± S.E.M., $n = 3$ (except at day 0, in which $n = 2$).

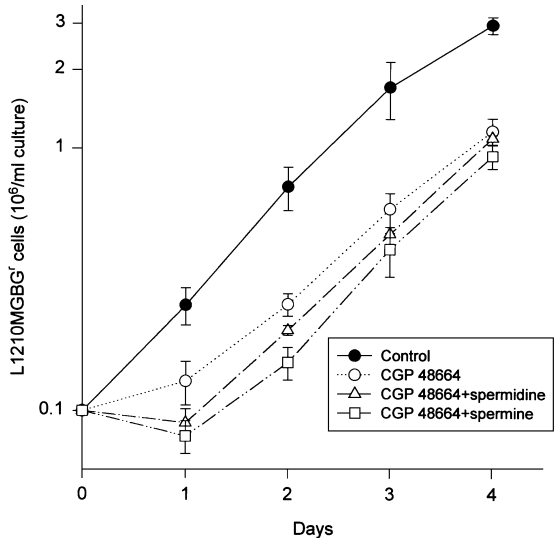

Figure 7 Effects of CGP 48664 on L1210-MGBG^r cell growth

Cells were seeded in the absence or presence of 2 μM CGP 48664 in combination with 2 μM spermidine or 1 μM spermine. Cells were counted daily. Symbols: ●, control cells; (○, cells grown in the presence of CGP 48664; △, cells grown in the presence of CGP 48664 and spermidine; □, cells grown in the presence of CGP 48664 and spermine. Each value is the mean ± S.E.M., $n = 3$ to 6.

Table 1 Effects of CGP 48664 on spermidine uptake in L1210 cells

The cellular uptake of 2 μM [^{3}H]spermidine was measured 1 day after seeding. The effect of CGP 48664 on spermidine uptake was determined after addition of various concentrations (0–400 μM) of the inhibitor to the assay. Each value is the mean ± S.D., $n = 3$.

[CGP 48664] (μM)	Spermidine uptake (pmol/h per 10^6 cells)
0 (control)	45.5 ± 1.5
4	45.2 ± 4.1
40	37.1 ± 1.8
400	13.7 ± 0.4

inhibition exerted by CGP 48664 was fully reversible by the addition of 2 μM spermidine or 1 μM spermine, strongly suggesting that the antiproliferative effect was due to the inhibition of AdoMetDC and thus of spermidine and spermine depletion (Figure 6).

Being an MGBG analogue it is conceivable that CGP 48664 enters the cells by the same transport system as the polyamines. To determine whether this was the case we used a mutant cell line, L1210-MGBG^r, isolated by selection for resistance against the cytotoxic effects of MGBG. These cells are deficient in the transport of various polyamines [32]. As shown in Figure 7, CGP 48664 exhibited an antiproliferative effect on L1210-MGBG^r cells that was of the same order of magnitude as that on the parental L1210 cells. In contrast with the effect on L1210 cells, the antiproliferative effect on the L120-MGBG^r cells was not reversed by the addition of spermidine or spermine (Figure 7). Furthermore, CGP 48664 was demonstrated to be a very poor competitor of spermidine uptake in the L1210 cells (Table 1), indicating that the inhibitor was not taken up by the polyamine transport system.

DISCUSSION

Inhibition of polyamine biosynthesis under both clinical and experimental conditions has usually been achieved by the use of ODC inhibitors. The most frequently used ODC inhibitor is the enzyme-activated irreversible inhibitor 2-difluoromethylornithine [6]. Treatment with 2-difluoromethylornithine usually results in a depletion of cellular putrescine and spermidine levels. The spermine content, however, generally remains unchanged or even increases. Spermidine has been argued to be more important than spermine in sustaining cell growth [5]. Because most cells have the ability to convert spermine into spermidine by using an interconversion pathway [33], inhibition of AdoMetDC might be a more powerful means of inhibiting both spermidine and spermine production and thus cell growth. However, until now all of the AdoMetDC inhibitors used have had some major disadvantages, such as non-specific effects, poor uptake or low stability [5,7]. CGP 48664 has been reported to be a much more potent and specific inhibitor of AdoMetDC than the previously used inhibitors of this enzyme [25]. It has also been demonstrated to exert a strong antitumour effect on various experimental tumours [25].

In the present study we demonstrate that CGP 48664 effectively inhibits spermidine and spermine synthesis in L1210 cells. In fact the total cellular amounts of spermidine and spermine in the cell culture remain virtually unchanged during the whole experimental period, indicating a complete block between putrescine and spermidine (results not shown). In spite of the total inhibition of spermidine and spermine production, the cells continued to grow, albeit at a slower rate. The reason for this is not known. Besides the cellular depletion of spermidine and spermine, the inhibitor gave rise to a large increase in the putrescine content. It is conceivable that putrescine at these high concentrations might replace spermidine and spermine in their growth-promoting functions, although less effectively. A similar phenomenon has been observed by using another AdoMetDC inhibitor, 5′-[(*Z*)-4-amino-2-butenyl]methylamino-5′-deoxyadenosine [22,34]. However, in that case a much stronger antiproliferative effect was obtained after a longer treatment with the inhibitor [34], indicating that the cells were slowly depleted of a factor important for growth. Results obtained indicate that the factor is the eukaryotic translation initiation factor 5A (eIF-5A) [34,35]. This initiation factor is the only known protein to contain the amino acid hypusine, which is formed by post-translational modification of a lysine residue by using an aminobutyl moiety derived from spermidine [36]. It is highly likely that the production of the eIF-5A will be hampered in cells depleted of their spermidine. That long-term exposure to CGP 48664 has a major deleterious effect, which is polyamine-related, on cell growth is indicated by the finding that chronic treatment of Chinese hamster ovary cells with increasing levels of CGP 48664 results in resistant cells that overproduce AdoMetDC owing to gene amplification [37].

The polyamines exert very strong feedback regulation of both ODC and AdoMetDC [3]. The compensatory increase in ODC synthesis observed after treatment with CGP 48664 was most probably related to this feedback control of the enzyme. As in other experimental systems [3], this feedback control of ODC synthesis seemed to be at the level of translation, because no change in the cellular amount of ODC mRNA was observed after treatment with the AdoMetDC inhibitor. Also, the synthesis of AdoMetDC was stimulated by treatment with CGP 48664. However, in contrast with the feedback control of ODC synthesis, the increase in AdoMetDC synthesis was partly explained by a rise in the amount of AdoMetDC mRNA, indicating an effect also on the transcription or turnover of the mRNA. Nevertheless

the major effect seemed to be at the translational level, because the increase in AdoMetDC mRNA was only 2-fold after treatment with CGP 48664, whereas the increase in synthesis rate was more than 8-fold.

AMG, being an inhibitor of copper-containing amine oxidases, is frequently used in cellular systems to prevent the oxidation of added polyamines to toxic aldehydes by the amine oxidase present in bovine serum [38]. However, as shown in the present study, AMG also affects AdoMetDC by giving rise to a marked stabilization of the enzyme. The half-life of the AdoMetDC protein, measured by a radioimmunoassay, was approx. 20 h when the cells were grown in the presence of AMG, whereas it was only 2–3 h when the cells were grown in the absence of AMG. However, in agreement with an earlier report [39], we found that the effect of AMG on the turnover of AdoMetDC activity was much smaller. Thus it seems that most of the AdoMetDC molecules were enzymically inactive in the presence of AMG. It is conceivable that AMG, perhaps by a transamination of the pyruvate at the active site, irreversibly inactivates AdoMetDC and that this inactive form of the enzyme is degraded much more slowly than the unmodified AdoMetDC. CGP 48664 and MGBG, which both have been shown to stabilize AdoMetDC against degradation ([17], and the present study), have one or two AMG-like moieties, and it is conceivable that the mechanism behind their potential as AdoMetDC inhibitors is closely related to the effects of AMG on AdoMetDC.

This study was supported by grants from the Swedish Cancer Society, the Medical Faculty (University of Lund), the Swedish Society for Medical Research, the IngaBritt and Arne Lundberg, the Dr. P. Håkansson, the Crafoord, the Wiberg, the Gyllenstiernska Krapperup, and the Gunnar, Arvid and Elisabeth Nilsson foundations. The investigation is part of EEC Concerted Action No. AIR1-CT-0569.

REFERENCES

1 Tabor, H. and Tabor, C. W. (1984) Annu. Rev. Biochem. **53**, 749–790
2 Pegg, A. E. (1988) Cancer Res. **48**, 759–774
3 Heby, O. and Persson, L. (1990) Trends Biochem. Sci. **15**, 153–158
4 Jänne, J., Alhonen, L. and Leinonen, P. (1991) Ann. Med. **23**, 241–259
5 Marton, L. J. and Pegg, A. E. (1995) Annu. Rev. Pharmacol. Toxicol. **35**, 55–91
6 McCann, P. P. and Pegg, A. E. (1992) Pharmacol. Ther. **54**, 195–215
7 Pegg, A. E. and McCann, P. P. (1992) Pharmacol. Ther. **56**, 359–377
8 Pajunen, A., Crozat, A., Jänne, O. A., Ihalainen, R., Laitinen, P. H., Stanley, B., Madhubala, R. and Pegg, A. E. (1988) J. Biol. Chem. **263**, 17040–17049
9 Stanley, B. A., Pegg, A. E. and Holm, I. (1989) J. Biol. Chem. **264**, 21073–21079
10 Pegg, A. E. (1977) FEBS Lett. **84**, 33–36
11 Demetriou, A. A., Cohn, M. S., Tabor, C. W. and Tabor, H. (1978) J. Biol. Chem. **253**, 1684–1686
12 Kameji, T. and Pegg, A. E. (1987) Biochem. J. **243**, 285–288
13 Stanley, B. A. and Pegg, A. E. (1991) J. Biol. Chem. **266**, 18502–18506
14 Svensson, F. and Persson, L. (1996) Mol. Cell. Biochem. **162**, 113–119
15 Pegg, A. E. and Williams-Ashman, H. G. (1968) Biochem. Biophys. Res. Commun. **30**, 76–82
16 Williams-Ashman, H. G. and Seidenfeld, J. (1986) Biochem. Pharmacol. **35**, 1217–1225
17 Pegg, A. E., Corti, A. and Williams-Ashman, H. G. (1973) Biochem. Biophys. Res. Commun. **52**, 696–701
18 Pegg, A. E. and McGill, S. M. (1978) Biochem. Pharmacol. **27**, 1625–1629
19 Pegg, A. E., Erwin, B. G. and Persson, L. (1985) Biochim. Biophys. Acta **842**, 111–118
20 Pegg, A. E., Jones, D. B. and Secrist, J. A. (1988) Biochemistry **27**, 1408–1415
21 Kramer, D. L., Khomutov, R. M., Bukin, Y. V., Khomutov, A. R. and Porter, C. W. (1989) Biochem. J. **259**, 325–331
22 Stjernborg, L., Heby, O., Mamont, P. and Persson, L. (1993) Eur. J. Biochem. **214**, 671–676
23 Wu, Y. Q. and Woster, P. M. (1995) Biochem. Pharmacol. **49**, 1125–1133
24 Regenass, U., Caravatti, G., Mett, H., Stanek, J., Schneider, P., Müller, M., Matter, A., Vertino, P. and Porter, C. W. (1992) Cancer Res. **52**, 4712–4718
25 Regenass, U., Mett, H., Stanek, J., Mueller, M., Kramer, D. and Porter, C. W. (1994) Cancer Res. **54**, 3210–3217
26 Persson, L. (1982) Acta Chem. Scand. **B36**, 685–688
27 Persson, L., Stjernborg, L., Holm, I. and Heby, O. (1989) Biochem. Biophys. Res. Commun. **160**, 1196–1202
28 Grens, A., Steglich, C., Pilz, R. and Scheffler, I. E. (1989) Nucleic Acids Res. **17**, 10497
29 Svensson, F., Kockum, I. and Persson, L. (1993) Mol. Cell. Biochem. **124**, 141–147
30 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **132**, 156–159
31 Holm, I., Persson, L., Pegg, A. E. and Heby, O. (1989) Biochem. J. **261**, 205–210
32 Ask, A., Persson, L. and Heby, O. (1992) Cancer Lett. **66**, 29–34
33 Casero, Jr., R. A. and Pegg, A. E. (1993) FASEB J. **7**, 653–661
34 Byers, T. L., Ganem, B. and Pegg, A. E. (1992) Biochem. J. **286**, 717–724
35 Byers, T. L., Wiest, L., Wechter, R. S. and Pegg, A. E. (1993) Biochem. J. **290**, 115–121
36 Joe, Y. A. and Park, M. H. (1994) J. Biol. Chem. **269**, 25916–25921
37 Kramer, D., Mett, H., Evans, A., Regenass, U., Diegelman, P. and Porter, C. W. (1995) J. Biol. Chem. **270**, 2124–2132
38 Zeller, A. E. (1963) in The Enzymes (Boyer, P. D., Lardy, H. and Myrbäck, K., eds.), vol. 8B, pp. 313–335, Academic Press, New York
39 Stjernborg, L. and Persson, L. (1993) Biochem. Pharmacol. **45**, 1174–1176

Received 4 July 1996/3 October 1996; accepted 15 October 1996