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Stimulation of Ornithine Decarboxylase Activity and Inhibition of *S*-Adenosyl-L-methionine Decarboxylase Activity in Leukaemic Mice by Methylglyoxal Bis(guanylhydrazone)

By OLLE HEBY,* SHARON SAUTER† and DIANE H. RUSSELL†‡
**Department of Neurological Surgery, University of California Medical Center, San Francisco, Calif. 94122, and †Department of Pharmacology, University of Arizona Medical Center, Tucson, Ariz. 85724, U.S.A.*

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Administration of methylglyoxal bis(guanylhydrazone) to leukaemic mice results in an early depression followed by a marked elevation of *S*-adenosyl-L-methionine decarboxylase activity. Further, there is an early prolonged increase in the activity of ornithine decarboxylase, the initial enzyme in the polyamine biosynthetic pathway. Because of the profound effects of methylglyoxal bis(guanylhydrazone) *in vivo* on the polyamine biosynthetic pathway, the drug can no longer be considered a specific inhibitor of spermidine synthesis.

Increased biosynthesis and subsequent accumulation of the polyamines putrescine, spermidine and spermine have been shown to occur in tissues after various growth stimuli (Dykstra & Herbst, 1965; Raina *et al.*, 1966; Russell & Snyder, 1968; Russell, 1970). Although the precise roles of the polyamines in growth processes are yet unknown, it has been postulated that they play a role in the control of RNA metabolism (Cohen, 1971). In a number of animal systems, the accumulation of spermidine parallels that of RNA (Caldarera *et al.*, 1965; Raina *et al.*, 1966; Russell & Lombardini, 1971; Heby & Lewan, 1971; Russell & McVicker, 1972). However, it is exceedingly difficult to obtain definitive data which specifically show that depression of spermidine synthesis leads to a depression of RNA synthesis. For this reason, the report by Williams-Ashman & Schenone (1972), which indicated that methylglyoxal bis(guanylhydrazone) was a potent inhibitor of spermidine synthesis *in vitro*, was received with great interest.

Several years ago it was reported that the anticancer effects of methylglyoxal bis(guanylhydrazone) could be prevented in L1210 leukaemic mice by the concurrent administration of spermidine (Mihich, 1963). Probably these results are due to inhibition of spermidine and spermine synthesis, since low concentrations of methylglyoxal bis(guanylhydrazone) inhibit putrescine-stimulated *S*-adenosyl-L-methionine decarboxylase in extracts of mammalian tissues (Williams-Ashman & Schenone, 1972). The Mg^{2+}-activated and putrescine-insensitive *S*-adenosyl-L-methionine decarboxylase of *Escherichia coli*, on the other hand, was inhibited only at very high concentrations of methylglyoxal bis(guanylhydrazone).

When studying the effects of various antineoplastic agents on the concentrations of putrescine, spermidine and spermine in spleens of leukaemic mice, we observed that all the drugs tested, with the exception of methylglyoxal bis(guanylhydrazone), caused decreases in putrescine, spermidine and spermine concentrations (Heby & Russell, 1973*a*). Methylglyoxal bis(guanylhydrazone) caused an almost 3-fold increase in the putrescine concentration but a decrease in the concentrations of spermidine and spermine. Accordingly, Fillingame & Morris (1973*a*) found that addition of methylglyoxal bis(guanylhydrazone) to transforming lymphocyte cultures led to almost complete suppression of both spermidine and spermine formation, whereas putrescine continued to accumulate, at an even higher rate. Surprisingly, the activities of both ornithine decarboxylase and *S*-adenosyl-L-methionine decarboxylase in dialysed extracts of the lymphocytes were found to be elevated (Fillingame & Morris, 1973*a*).

In an attempt to determine the cause of putrescine accumulation in methylglyoxal bis(guanylhydrazone)-treated leukaemic mice we have studied the activities of the polyamine biosynthetic enzymes in spleen extracts of leukaemic and non-leukaemic mice at various times after a single injection of the drug.

Materials and methods

Chemicals. DL-[1-^{14}C]Ornithine monohydrochloride (12.8mCi/mmol) and *S*-adenosyl-L-[*carboxy*-^{14}C]-methionine (7.7mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. Pyridoxal 5-phosphate, dithiothreitol and the hydrochloride salts of putrescine and spermidine were purchased from Calbiochem, Gaithersburg, Md., U.S.A. Methylglyoxal bis(guanylhydrazone) (NSC-32946) was obtained from Microbiological Associates, Bethesda, Md., U.S.A., through the courtesy of Dr. Harry B. Wood, Jr., National Cancer Institute, Bethesda, Md., U.S.A.

Experimental animals. BDF_1 male mice and lymphoid leukaemia L1210 tumour strain were obtained

‡ To whom reprint requests should be addressed.

from Microbiological Associates. Experimental details of tumour maintenance and transfer have been previously described (Heby & Russell, 1973*b*). Into each mouse 10^6 L1210 ascites-tumour cells were inoculated intraperitoneally.

Drug regimen. At 6 days after tumour inoculation, methylglyoxal bis(guanylhydrazone) (50mg/kg) in 0.9% NaCl, or 0.9% NaCl alone, was injected intraperitoneally in a total volume of 0.1ml.

Enzyme assays. The spleens of leukaemic and non-leukaemic mice were excised, chilled on ice, rinsed in 0.9% NaCl, blotted, weighed, and immediately homogenized in 5vol. of ice-cold 0.05M-sodium-potassium phosphate buffer, pH7.2, containing 1mM-dithiothreitol. Because any methylglyoxal bis(guanylhydrazone) still present in the spleen extracts would directly inhibit the *S*-adenosyl-L-methionine decarboxylase activities (Heby & Russell, 1973*a*), the supernatant after centrifugation at 20000*g* was dialysed for 4h in three changes of 3 litres of 0.01M-sodium-potassium phosphate buffer, pH7.2, containing 0.01mM-dithiothreitol and 3μM-pyridoxal 5-phosphate. Dialysed supernatants were used as enzyme preparations in all the assays.

Assay for ornithine decarboxylase activity. Enzyme activity was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine as previously described (Russell & Snyder, 1968) with the following minor modification. The buffer used in the assays was 0.05M-sodium-potassium phosphate buffer, pH7.2, containing 1mM-dithiothreitol.

Assays for putrescine- or spermidine-stimulated S-adenosyl-L-methionine decarboxylase activities. Enzyme activities were determined by measuring the release of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxy*-^{14}C]-methionine in the presence of either putrescine or spermidine, as previously described (Russell, 1972). The activators putrescine or spermidine were added in saturating concentrations, i.e. 2.5mM and 5mM respectively.

Results

We have studied the effects of a single intraperitoneal injection of methylglyoxal bis(guanylhydrazone) on polyamine biosynthesis in the spleen of normal and leukaemic mice 6 days after the inoculation of L1210 cells. At 6 days after transplantation the leukaemic infiltration almost completely obliterated the normal structure of the spleen (Heby & Russell, 1973*a*).

Within 4h after administration of methylglyoxal

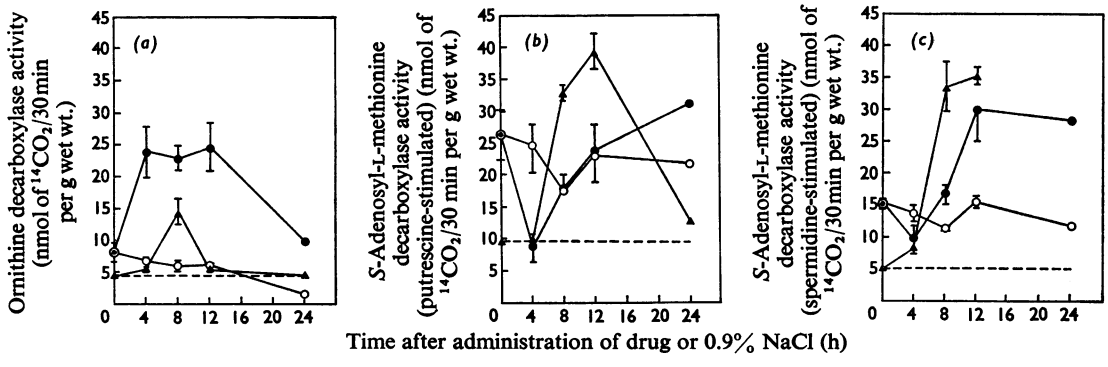

Fig. 1. *Changes in the activities of the enzymes in the polyamine biosynthetic pathway in the mouse spleen in response to administration of methylglyoxal bis(guanylhydrazone) (50mg/kg)*

Methylglyoxal bis(guanylhydrazone) in 0.9% NaCl, or 0.9% NaCl alone, was administered in a total volume of 0.1ml. Tumour-bearing mice had been inoculated with 10^6 L1210 leukaemic cells 6 days before initiation of methylglyoxal bis(guanylhydrazone) treatment. Previously it has been shown that by day 6 of tumour growth leukaemic infiltration almost completely obliterates the normal structure of the spleen (Heby & Russell, 1973*a*). (*a*) Ornithine decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine. (*b*) Putrescine-stimulated and (*c*) spermidine-stimulated *S*-adenosyl-L-methionine decarboxylase activities were determined by measuring the release of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxy*-^{14}C]methionine in the presence of putrescine or spermidine respectively. Each value plotted represents the mean±S.E.M. of four spleen extracts. Five spleens were used for the preparation of each extract. o, Tumour-bearing mice given 0.9% NaCl. ●, Tumour-bearing mice given methylglyoxal bis(guanylhydrazone). ----, Control (non-tumour) mice given 0.9% NaCl. ▲, Control (non-tumour) mice given methylglyoxal bis(guanylhydrazone). In all cases enzyme activities were measured at 4, 8, 12 and 24h after administration of 0.9% NaCl or the drug.

bis(guanylhydrazone) ornithine decarboxylase activity increased 3-fold in leukaemic mice (Fig. 1*a*). This marked elevation of ornithine decarboxylase activity persisted through 12h after administration of methylglyoxal bis(guanylhydrazone). The enzyme activity then declined, but was still elevated by 24h after injection of methylglyoxal bis(guanylhydrazone) when compared with the controls (0.9%-NaCl-injected leukaemic mice). In non-leukaemic mice there was a brief, but 3-fold, increase in the ornithine decarboxylase activity at 8h after administration of methylglyoxal bis(guanylhydrazone) when compared with the controls (0.9%-NaCl injected non-leukaemic mice). Addition of methylglyoxal bis(guanylhydrazone) directly to the reaction mixture did not markedly affect the ornithine decarboxylase activity.

The putrescine-stimulated *S*-adenosyl-L-methionine decarboxylase activity in leukaemic mice was markedly depressed 4h after administration of methylglyoxal bis(guanylhydrazone) when compared with the controls (0.9%-NaCl-injected leukaemic mice) even after extensive dialysis (Fig. 1*b*). At 8–12h after the injection of methylglyoxal bis(guanylhydrazone), however, the activities of putrescine-stimulated *S*-adenosyl-L-methionine decarboxylase were similar to those of the controls (0.9%-NaCl-injected leukaemic mice). Within 24h after drug administration the enzyme activity was significantly elevated. The putrescine-stimulated *S*-adenosyl-L-methionine decarboxylase activity in non-leukaemic mice showed a 4-fold increase within 12h after administration of methylglyoxal bis(guanylhydrazone) when compared with the controls (0.9%-NaCl-injected non-leukaemic mice) (Fig. 1*b*). A decrease in the enzyme activity at 4h was recorded only if the enzyme preparation was not dialysed. The increase in enzyme activity was transient, and 24h after drug treatment the activity was within the range of the control.

The pattern of spermidine-stimulated *S*-adenosyl-L-methionine decarboxylase activity after administration of methylglyoxal bis(guanylhydrazone) (Fig. 1*c*) was similar to that of the putrescine-stimulated activity (Fig. 1*b*) for all four groups of mice. However, at 8–12h after the drug injection the spermidine-stimulated *S*-adenosyl-L-methionine decarboxylase activity in leukaemic mice was significantly elevated over that of the controls (0.9%-NaCl-injected leukaemic and non-leukaemic mice). In the control groups (0.9%-NaCl-injected leukaemic and non-leukaemic mice) there was about a 2:1 preference for putrescine compared with spermidine as the acceptor for the propylamine moiety from decarboxylated *S*-adenosyl-L-methionine at all times (Figs. 1*b* and 1*c*). However, in the methylglyoxal bis(guanylhydrazone)-treated animals the enzyme no longer exhibited any preference for putrescine as opposed to spermidine, i.e. the activity of spermidine-stimulated *S*-adenosyl-L-methionine decarboxylase was as high as that of the putrescine-stimulated enzyme. This observation indicates that methylglyoxal bis(guanylhydrazone) may change the specificity requirements for substrate.

Discussion

During the last few years it has been shown repeatedly that very low concentrations (<1 μM) of methylglyoxal bis(guanylhydrazone) almost completely inhibit *S*-adenosyl-L-methionine decarboxylase activities when added to enzyme preparations or cell-culture media, or after injection into animals (Williams-Ashman & Schenone, 1972; Heby & Russell, 1973*a*; Fillingame & Morris, 1973*a*,*b*; Pegg, 1973; Kay & Pegg, 1973; Pegg *et al.*, 1973). The increase in the putrescine concentration produced by the inhibitor seemed to be caused by the continued formation of putrescine by ornithine decarboxylase while synthesis of spermidine and spermine was inhibited. Ornithine decarboxylase activity was reported not to be affected when methylglyoxal bis(guanylhydrazone) was added directly to the tissue extracts. However, in accordance with a previous observation (Fillingame & Morris, 1973*a*) we have found that treatment with methylglyoxal bis(guanylhydrazone) *in vivo* markedly stimulates ornithine decarboxylase activity. Therefore the accumulation of putrescine subsequent to methylglyoxal bis(guanylhydrazone) treatment seems to be due not only to inhibition of spermidine synthesis but also to increased synthesis of putrescine. Strangely enough, however, *S*-adenosyl-L-methionine decarboxylase activities were also markedly elevated after methylglyoxal bis(guanylhydrazone) treatment when the enzyme was assayed in dialysed tissue extracts (Fillingame & Morris, 1973*a*; Pegg *et al.*, 1973). Recent results indicate that the enhancement of *S*-adenosyl-L-methione decarboxylase activities after administration of methylglyoxal bis(guanylhydrazone) may be due to an effect of this drug on the half-life of this enzyme (Pegg *et al.*, 1973; Fillingame & Morris, 1973*b*). The half-life increased from 2h to more than 20h in the kidney and from 40min to more than 20h in concanavalin A-stimulated lymphocytes after administration of methylglyoxal bis(guanylhydrazone). It was suggested that the drug decreases the degradation of *S*-adenosyl-L-methionine decarboxylase by direct interaction with the enzyme (Fillingame & Morris, 1973*b*). Assays of non-dialysed spleen extracts showed that the *S*-adenosyl-L-methionine decarboxylase activities were greatly inhibited for several hours after treatment with methylglyoxal bis(guanylhydrazone). Also, since the assay conditions represented a 5-fold dilution of the tissues the inhibition of the enzyme *in vivo* may have been greater than that evaluated *in vitro*.

The stimulation of ornithine decarboxylase activity as well as the inhibition of *S*-adenosyl-L-methionine

decarboxylase activity would account for the increased concentration of putrescine found in tissues after administration of methylglyoxal bis(guanylhydrazone) and indicate that any attempt to use this drug as a tool to ascertain the precise role that spermidine plays in mammalian tissues will have to be interpreted with caution. That is, severalfold increases in the putrescine concentrations could well be sufficient to allow for normal cellular function in spite of decreased synthesis of spermidine and spermine.

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