# EFFECT OF INHIBITION OF SPERMIDINE FORMATION ON PROTEIN AND NUCLEIC ACID SYNTHESIS DURING LYMPHOCYTE ACTIVATION

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## 1. Introduction

In many types of mammalian cells the activities of the enzymes involved in polyamine synthesis increase very markedly with the growth rate, and the polyamine content correlates well with the RNA content [1]. While a variety of effects of polyamines on protein and nucleic acid synthesis in mammalian cells, and cell-free systems derived from them, have been reported [1, 2], their biological function is still far from clear. Perhaps the most favoured suggestion is that they may be involved in the control of ribosomal RNA synthesis [3, 4], which is itself intimately connected with the growth rate.

Methyl glyoxal bis (guanylhydrazone) (MGBG) is a potent inhibitor of the putrescine-dependent S-adenosyl methionine decarboxylase isolated from yeast and animal cells [5], and thus should prevent the synthesis of spermidine from putrescine [6]. Injection of this drug causes a depression of the growth of leukaemic cells in mice that is prevented by simultaneous injection of spermidine [7]. We have used this inhibitor to study the requirement for spermidine synthesis during the activation of cultured human lymphotyces by phytohaemagglutinin (PHA).

Human lymphocytes do not normally synthesize DNA or divide in culture, but addition of PHA to the culture medium induces them to initiate DNA synthesis after a lag of about 30 hr [8]. A number of metabolic changes occur prior to the initiation of DNA synthesis, including increases in the rates of RNA and protein synthesis. The increase in the rate of synthesis of ribosomal RNA is especially marked [9]. Prominent amongst these early changes are marked rises in the activities of the polyamine synthesizing enzymes, which are present at very low levels in unstimulated lymphocytes [10, 11]. S-adenosyl methionine decarboxylase activity begins to rise about 4 hr after the addition of PHA, and is followed by the rise in ornithine decarboxylase activity [11]. Low concentrations of MGBG strongly and specifically inhibit S-adenosyl methionine decarboxylase activity in lymphocyte extracts and prevent spermidine synthesis in intact lymphocytes. Stimulation of lymphocytes by PHA in the presence of the drug has shown that the induction of this enzyme is more directly associated with the stimulation of protein synthesis than with ribosomal RNA synthesis, which increases at the normal rate for about 18 hr.

## 2. Materials and methods

Lymphocytes were purified from human blood as described by Cooper [12] and incubated at $2 \times 10^6$/ml in Eagle's minimal essential medium supplemented with 10% autologous plasma. PHA-Q1, Wellcome Research Laboratories, Beckenham, Kent, was added where indicated to a final conc. of 4 $\mu$g/ml. MGBG was purchased from Aldrich Chemical Co., Milwaukee, Wisc., spermidine from Sigma Chemical Co., St. Louis, Mo., and radiochemicals from the Radiochemical Centre, Amersham, Bucks.

To determine conversion of [$^{14}$C] putrescine to spermidine and spermine, cultures containing $10^7$ lymphocytes were incubated with 0.5 $\mu$Ci/ml [1,4-$^{14}$C]-putrescine (17.8 mCi/mmole). The cells were then washed twice with 0.15 M NaCl, and proteins and

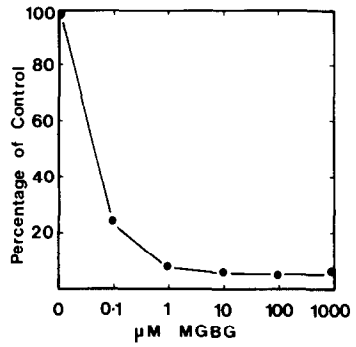


Fig. 1. Effect of MGBG on S-adenosyl methionine decarboxylase activity of extracts of lymphocytes incubated with PHA for 42 hr. MGBG was added to the extracts immediately before the substrate.

Table 1

Conversion of [$^{14}$C] putrecine to polyamines by lymphocytes incubated with PHA and with or without 1 mM MGBG. Incubation conditions are described under Materials and methods. Results are expressed as dpm/$10^6$ lymphocytes.

| Incubation (hr) | MGBG | Putrescine | Spermidine | Spermine |
|---|---|---|---|---|
| 6 | – | 3769 | 705 | 44 |
| 6 | + | 2097 | 26 | 14 |
| 15 | – | 4095 | 1772 | 169 |
| 15 | + | 2071 | 36 | 23 |

nucleic acids precipitated with 2 ml 5% $CCl_3COOH$. Putrescine and polyamines were recovered from the supernatant extract, resolved by high voltage electrophoresis and counted as previously described [13].

The methods used for the assay of S-adenosyl methionine decarboxylase activity in lymphocyte extracts [11], for extraction of lymphocyte RNA by the cold phenol method and its analysis by sucrose gradient centrifugation [14], and for the determination of the rates of incorporation of [$^3$H] uridine into RNA, [$^3$H] thymidine into DNA and [$^{14}$C] phenylalanine into protein [11] were as previously described.

## 3. Results

MGBG inhibited S-adenosyl methionine decarboxylase activity in both lymphocyte extracts and intact lymphocytes. In extracts 75% inhibition was found at

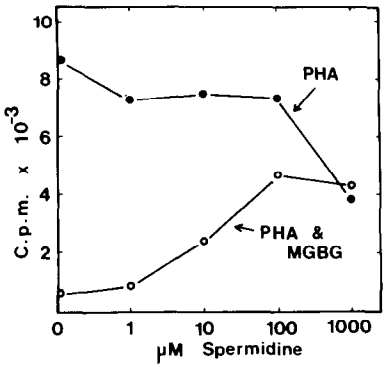


Fig. 2. Reversal by spermidine of the inhibition by MGBG of [$^{14}$C] phenylalanine incorporation into protein. Spermidine and MGBG were added at the same time as PHA to cultures containing $10^6$ lymphocytes. After incubation for 46 hr, 1 μCi [U-$^{14}$C] phenylalanine was added to each culture, and incorporation was determined after a further 2 hr.

0.1 μM MGBG and more than 90% inhibition at 1 μM (fig. 1). The conversion of added [$^{14}$C] putrescine to spermidine by lymphocytes was also almost completely inhibited by MGBG (table 1). The uptake of [$^{14}$C] putrescine was inhibited by about 50% by 1 mM MGBG, possibly due to the accumulation of endogenous putrescine in the presence of the drug.

Preliminary experiments showed that addition of MGBG to lymphocytes at the same time as PHA resulted in essentially complete inhibition of the stimulation of incorporation of [$^3$H] thymidine into DNA and [$^{14}$C] phenylalanine into protein 48 hr later. Inhibition of thymidine incorporation occurred with MGBG concentrations down to 10 μM. The effects of even high concentrations of MGBG could be reversed by addition of spermidine to the cultures (fig. 2), showing that the action of the drug was most probably due to its effects on polyamine metabolism, rather than side effects. The effects of MGBG could also be reversed by washing the cultures free of the drug up to 24 hr after its addition.

The effects of MGBG added together with PHA on the early stimulation of the incorporation of [$^3$H] uridine into RNA and [$^{14}$C] phenylalanine into protein are shown in fig. 3. Initially PHA stimulated the incorporation of both precursors in the presence of the drug. Inhibition of [$^{14}$C] phenylalanine incorporation became apparent first, and by about 30 hr the rate of incorporation had returned to near the basal level. The

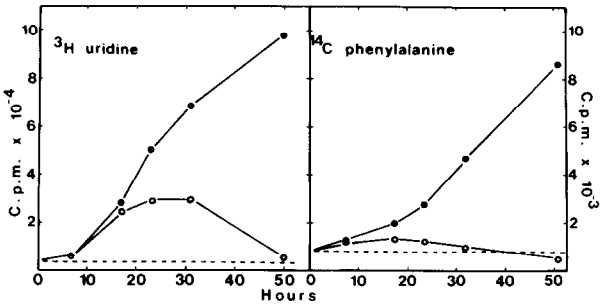


Fig. 3. Effect of 1 mM MGBG added together with PHA on the incorporation of [$^{3}$H]uridine into RNA and [$^{14}$C]phenylalanine into protein. Each culture contained $10^{6}$ lymphocytes, and incorporation was determined after incubation with 1 μCi [U-$^{14}$C]phenylalanine for 2 hr or 10 μCi [5-$^{3}$H]uridine for 1 hr. (●—●—●) PHA only. (○—○—○) PHA and MGBG. (– – –) Unstimulated lymphocytes.

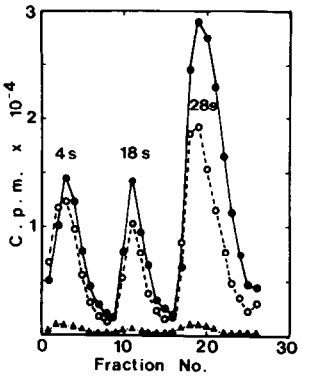


Fig. 4. Effect of 1 mM MGBG on the types of RNA synthesized. Cultures containing 25 × $10^{6}$ lymphocytes were incubated with 1 μCi/ml [5-$^{3}$H]uridine, and with or without PHA and 1 mM MGBG, for 22 hr. RNA was extracted and analysed by sedimentation through 5–20% sucrose in 0.1 M NaCl, 0.01 M $CH_3COONa$ pH 5.1, at 50,000 rpm for 2.5 hr in the SW50L rotor. (●—●—●) PHA only. (○—○—○) PHA and MGBG. (▲—▲—▲) Unstimulated lymphocytes.

stimulation of [$^{3}$H]uridine incorporation into RNA was essentially normal for the first 18 hr. Little further increase occurred after 18 hr, but the rate of incorporation remained high at least a further 10 hr. Analysis of the RNA synthesized by sucrose gradient centrifugation showed that PHA caused a marked stimulation of the labeling of both 18 S and 28 S ribosomal RNA in the first 22 hr, whether or not MGBG was present in the incubation medium (fig. 4).

## 4. Discussion

The continued synthesis of ribosomal RNA when spermidine synthesis is inhibited by MGBG shows that the increased spermidine synthesis observed after lymphocyte activation is not obligatory for the increase in ribosomal RNA synthesis. Conversely, the increase in polyamine synthesis is not simply a consequence of increased ribosomal RNA synthesis, as the polyamine synthesizing enzymes are still induced when lymphocytes are stimulated by PHA in the presence of low doses of actinomycin, which selectively suppress ribosomal RNA synthesis [11].

Our results would be consistent with an essential role for the increased polyamine synthesis generally found associated with growth stimulation in mammalian cells being related to the increase in ribosomal RNA synthesis if putrescine were the important agent. We have not measured the accumulation of putrescine during the incubation of lymphocytes with MGBG, but in liver and kidney it increases markedly [15]. If putrescine controlled the rate of ribosomal RNA synthesis, a stimulation might be expected in cells incubated with MGBG. In addition, direct addition of putrescine to stimulated or unstimulated lymphocytes does not affect their rates of RNA or protein synthesis, although the putrescine can be shown to enter the cells (unpublished data).

It is conceivable that enough spermidine is initially present to allow the continued synthesis of ribosomal RNA observed, and that the inhibition after 18 hr is due to lack of spermidine. It is, however, much more likely that both this inhibition, and the prevention of the initiation of DNA synthesis, are secondary to inhibition of protein synthesis.

It is also possible that polyamines might be involved in ribosome function rather than ribosome synthesis, and that the inhibition of protein synthesis could follow from the synthesis of defective ribosomes. If this is the case, the defective ribosomes must actually be inhibitory rather than simply non-functional, as protein synthesis can continue to increase at nearly the normal rate for at least 18 hr when ribosome synthesis is completely prevented by the addition of low concentrations of actinomycin [16]. The inhibition of the increase in protein synthesis is the earliest event

that we have observed to follow the addition of MGBG. This, however, may itself follow from some as yet unidentified change, and it would be premature to conclude that polyamines are directly involved in the increase in protein synthesis.

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