EFFECT OF METHYLGLYOXAL BIS(GUANYLHYDRAZONE)* ON *S*-ADENOSYLMETHIONINE DECARBOXYLASE IN THE ISOLATED PERFUSED RAT LIVER

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1. Introduction

S-Adenosylmethionine decarboxylase (E.C. 4.1.1.50) is a key enzyme in the biosynthetic pathway leading to polyamines [1–7]. The possibility that information as to the biological role of polyamines might be obtained by inhibition of the activity of this enzyme was raised by the discovery that methylglyoxal bis(guanylhydrazone) (MGBG) is a very potent but reversible inhibitor of mammalian *S*-adenosylmethionine decarboxylases [1]. However studies of the effects of this inhibitor in vivo have revealed that its administration leads to a large increase in the amount of *S*-adenosylmethionine decarboxylase activity present in various tissues when extracts are assayed removal of any residual inhibitor [2–5]. This finding coupled with the toxicity and rate of excretion of the drug has prevented the use of MGBG to investigate the role of polyamines in intact animals, although studies of the effects of MGBG on cells in culture have proved more rewarding [5–7]. The mechanism by which a large increase in the activity of an enzyme inhibited by MGBG is produced by administration of the drug is of considerable interest particularly since MGBG is a useful antileukaemic agent [8]. The present paper reports studies of the effects of MGBG on *S*-adenosylmethionine decarboxylase activity in the isolated perfused liver. Perfusion of the liver with a medium containing MGBG led to an increase in the activity of *S*-adenosylmethionine decarboxylase and prevented the loss of enzyme activity produced by cycloheximide, but perfusion with putrescine was ineffective in these respects. These results indicate that the increased *S*-adenosylmethionine decarboxylase activity produced by the inhibitor may be due to stabilisation of the enzyme against intracellular degradation by combination with the inhibitor. They also rule out the possibility that the increased concentrations of putrescine (which is an activator of the *S*-adenosylmethionine decarboxylase) brought about by MGBG are responsible for the increased enzyme activity.

* The correct chemical name for this drug is 1,1'-[(methylethanediylidene)-dinitrilo] diguanidine.

2. Methods

Male Wistar rats weighing 100–115 g were used in all experiments. Livers were perfused in situ as previously described [9]. The basic perfusion medium consisted of Krebs–Henseleit bicarbonate buffer containing 3% (w/v) bovine serum albumin, 8 mM glucose, sufficient washed bovine erythrocytes to give a hematocrit of 20–22%, and unless indicated otherwise a mixture of 20 amino acids at ten times their concentration in normal rat plasma. The perfusate

was equilibrated with humidified O_2–CO_2 (95%:5%) at 37°C in rotating reservoirs and pumped through the liver at a constant rate of 7.0 ml/min.

S-Adenosylmethionine decarboxylase and ornithine decarboxylase activity were determined as previously described [10, 11]. Extracts from tissues which had been treated with MGBG were dialysed to remove any residual inhibitor [3] and all assays were conducted under conditions where the measured activity was proportional to the amount of tissue protein added and to the time of incubation.

Putrescine concentrations were determined by extraction of frozen tissue samples and electrophoretic separation of the amines present [11]. The recovery of putrescine during the extraction was monitored by the addition of trace amounts of radioactive putrescine to the extracts and was 80–90%. The values given were corrected for this loss.

3. Results and discussion

There was little change in the *S*-adenosylmethionine decarboxylase activity of livers perfused for 3 hr under standard conditions (table 1). However, if MGBG (0.1 mM) was present in the perfusion medium and the activity of the enzyme was assayed in tissue extracts which had been dialysed in order to remove the inhibitor, the activity was doubled within the 3 hr perfusion period. This result is in agreement with previous experiments where MGBG was administered to rats [2–4]. Such changes observed in rats in vivo might be mediated through some alterations in hormone levels brought about by MGBG since *S*-adenosylmethionine decarboxylase activity is known to be regulated by a variety of hormonal factors [9, 10]. However, it is unlikely that the inhibitor could bring about changes by such a mechanism in the isolated perfused liver.

Mammalian *S*-adenosylmethionine decarboxylases are known to be activated by putrescine [10]. Putrescine is formed in mammalian tissues by the action of ornithine decarboxylase and is converted into spermidine by the action of spermidine synthetase which uses decarboxylated *S*-adenosylmethionine as a source of the propylamine moiety needed for conversion of putrescine into spermidine [10–12]. When production of decarboxylated *S*-adenosylmethionine is blocked by MGBG, putrescine cannot be converted into spermidine and accumulates [2, 4, 6, 7]. Since putrescine is an activator of mammalian *S*-adenosylmethionine decarboxylases and stabilises the enzyme in vitro against attack by proteolytic enzymes [3], it is possible that the increased levels of putrescine resulting from treatment with MGBG lead to the increased activity of *S*-adenosylmethionine decarboxylase. However, as shown in table 1, perfusion of the liver with putrescine (6.2 mM) for 3 hr did not lead to an increase in the *S*-adenosylmethionine decarboxylase activity although this treatment did produce a

Table 1

Effect of perfusion with MGBG or putrescine on activity of *S*-adenosylmethionine decarboxylase and putrescine concentration in rat liver

	S-Adenosylmethionine decarboxylase activity (nmole CO_2/hr/g wet wt.)	Putrescine content (nmole/g wet wt.)
Control (not perfused)	42.5 ± 1.2 (34)	33 ± 3 (4)
Perfused for 3 hr	38.1 ± 1.2 (8)	32 ± 2 (5)
Perfused for 3 hr + 0.1 mM MGBG	83.0 ± 2.3 (5)	48 ± 6 (3)
Perfused for 3 hr + 6.2 mM putrescine	43.2 ± 2.0 (3)	742 ± 28 (3)

Results are given ± S.E.M. for the number of estimations shown. After perfusion of the livers with putrescine, the intracellular putrescine content of the liver was determined after a 10 min perfusion without putrescine in order to wash out extracellular putrescine. This procedure was shown to be effective by measurement of the loss from the liver of [^{3}H]putrescine. After 5 min of wash out, radioactivity of the effluent perfusate had fallen to less than 1% of that present during the recirculation period (2 μCi/ml) and by 10 min virtually no radioactivity was detectable in the effluent.

very substantial rise in the liver putrescine content. It is therefore unlikely that an increase in the intracellular putrescine concentration can be responsible for the MGBG-mediated increase in *S*-adenosylmethionine decarboxylase activity.

This interpretation is supported by analysis of the putrescine content of the livers perfused with MGBG (table 1). There was only a small increase in the concentration of putrescine during the perfusion although the *S*-adenosylmethionine decarboxylase activity was doubled. Considerably greater increases in liver putrescine content were observed 8 and 20 hr after administration of the inhibitor in vivo [2] but cannot be directly compared to the present results since the time of perfusion was only 3 hr. Also the fact that ornithine decarboxylase activity declines very sharply after perfusion of the liver is started and only slowly recovers (unpublished data) may prevent greater accumulation of putrescine during the perfusion with MGBG.

S-Adenosylmethionine decarboxylase activity is rapidly lost after the application of inhibitors of protein synthesis to intact animal or cells in culture [3, 5, 12, 13]. A similar rapid decline in the activity of this enzyme is seen after perfusion in the presence of cycloheximide (fig. 1). The apparent half-life of liver *S*-adenosylmethionine decarboxylase measured in this way is 65 min which is in reasonable agreement with that found in rat liver in vivo [13]. Perfusion in the presence of putrescine had no effect on the rate of loss of *S*-adenosylmethionine decarboxylase activity suggesting that the change in the intracellular content of putrescine from about 32 nmoles/g wet wt. to 742 nmoles/g wet wt. did not alter the rate of degradation of the enzyme to an inactive form. In contrast the inclusion of MGBG in the perfusion medium almost completely prevented the loss of activity during a 3 hr perfusion in the presence of cycloheximide (fig. 1). This effect was not due to a generalised inhibition of protein degradation by MGBG since ornithine decarboxylase activity which is also rapidly lost on perfusion of the liver with cycloheximide declined at a similar rate in the presence or absence of MGBG (fig. 1).

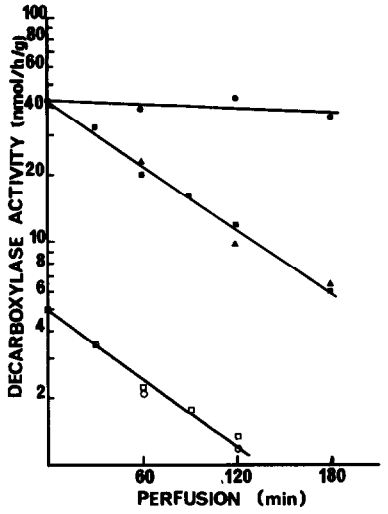

Fig. 1. Loss of activity of *S*-adenosylmethionine decarboxylase and ornithine decarboxylase after perfusion in the presence of cycloheximide. The perfusion medium contained 0.1 mM cycloheximide but no amino acids. Results are shown for *S*-adenosylmethionine decarboxylase activity in extracts from livers perfused with no addition (■), 0.1 mM MGBGB (●) and 6.2 mM putrescine (▲). Ornithine decarboxylase activity is presented for perfusions with no addition (□) and 0.1 mM MGBG (○).

These results indicate that MGBG can bring about an increase in *S*-adenosylmethionine decarboxylase activity in the isolated perfused liver and that this increase is not mediated through hormonal influences or through an increase in the tissue putrescine level. Although considerable care must be taken in equating changes in enzyme activity with changes in the amount of enzyme protein actually present, in many of the cases where this has been investigated by both methods the loss of enzyme activity seen in the presence of inhibitor has been found to correspond to changes in protein content [14]. In the present situation this remains to be established but the hypothesis that MGBG brings about the increase in *S*-adenosylmethionine decarboxylase activity by combining with the enzyme in such a way as to diminish the rate of degradation of the enzyme protein remains the most likely explanation of these results. The system described may therefore be of value in studying the processes underlying cellular protein degradation mechanisms.

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