Inhibition of Spermidine Formation in Rat Liver and Kidney by Methylglyoxal Bis(guanyldiazotane)

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The effect of methylglyoxal bis(guanyldiazotane), a substance known to inhibit putrescine-dependent *S*-adenosyl-L-methionine decarboxylase, on polyamine metabolism in liver and kidney was investigated. Almost complete inhibition of the incorporation of putrescine into spermidine was obtained up to 8 h after administration of 80 mg of methylglyoxal bis(guanyldiazotane)/kg body wt. by intraperitoneal injection. However, by 20 h after administration of the inhibitor spermidine synthesis was resumed. Considerable accumulation of putrescine occurred during this period (up to 3 times control concentrations in both tissues), but there was only a slight fall in the spermidine content. These results suggest that the putrescine-activated *S*-adenosyl-L-methionine decarboxylase plays an essential role in spermidine biosynthesis in rat liver and kidney, and the possibility of using methylglyoxal bis(guanyldiazotane) to study the role of polyamine synthesis in growth is discussed.

The functions of polyamines in cellular metabolism are not yet clearly understood, but it is now well established that an increased accumulation of polyamines is a feature of many situations in which increased tissue growth occurs. Such increases occur under a variety of stimuli including tissue regeneration (Dykstra & Herbst, 1965; Raina *et al.*, 1966; Jänne, 1967), hormonal induction (Kostyo, 1966; Jänne, 1967; Moulton & Leonard, 1969; Pegg *et al.*, 1970) and developmental growth (Raina, 1963; Caldarera *et al.*, 1965; Barros & Guidice, 1968; Russell, 1970). Polyamines may be an essential part of the ribosome complex and have been shown *in vitro* to stimulate protein synthesis, aminoacyl-tRNA synthetases, RNA polymerase and tRNA methylases (Tabor & Tabor, 1964; Stevens, 1970; Bachrach, 1970; Takeda & Igarashi, 1969; Leboy, 1970; Pegg, 1971). However, it is not known whether polyamines are obligatory cofactors for these reactions *in vivo*, and it is not clear to what extent the increased accumulation of polyamines is essential for the synthesis of macromolecules during growth. The important recent discovery that methylglyoxal bis(guanyldiazotane) is a potent inhibitor of the putrescine-dependent *S*-adenosyl-L-methionine decarboxylase of yeast and rat prostate (Williams-Ashman & Schenone, 1972) may provide a useful tool for answering this question, since this enzyme may be essential for spermidine synthesis by mammalian cells (Pegg & Williams-Ashman, 1969; Pegg, 1970; Jänne & Williams-Ashman, 1971). The aim of the present experiments was to determine whether methylglyoxal bis(guanyldiazotane) was able to enter the cells of rat liver and kidney and to inhibit the formation of spermidine in these tissues.

Experimental

S-Adenosyl-L-[carboxy-¹⁴C]methionine (61 mCi/mmol) and [1,4-¹⁴C₂]putrescine dihydrochloride (17.8 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Methylglyoxal bis(guanyldiazotane) was obtained from Aldrich Chemical Co., Milwaukee, Wis., U.S.A., and was dissolved in 0.14 M-NaCl before administration by intraperitoneal injection to male rats (weighing 130 g) of the Wistar stock bred in this Institute.

Polyamines were extracted from isolated rat tissue by the method of Raina (1963), separated by high-voltage electrophoresis (40 V/cm) in 0.1 M-sodium citrate buffer, pH 4.1, for 90 min and measured as previously described (Pegg *et al.*, 1970). Incorporation of labelled putrescine into spermidine and spermine was determined after the intraperitoneal injection of 0.01 mCi of [1,4-¹⁴C₂]putrescine (17.8 mCi/mmol) dissolved in 0.2 ml of 0.14 M-NaCl 4 h before death. Radioactivity present in each polyamine fraction was determined as described by Russell *et al.* (1970). The efficiency of counting the radioactivity of polyamine samples in the Packard Tri-Carb 3320 liquid-scintillation counter varied between 35 and 50% according to the colour and was determined for each sample by the addition of an internal standard of [¹⁴C]toluene (Packard Instrument Co., Chesham, Reading, Berks., U.K.). Results were expressed as nmol of each polyamine present per g of tissue (wet wt.) and d.p.m. present in each polyamine fraction from 1 g of tissue. No correction was made for losses during extraction which were found to be less than 8% in separate experiments.

Tissue samples were homogenized in 2.5 vol. of 0.01 M-potassium phosphate-0.1 mM-EDTA-1 mM-

2-mercaptoethanol, pH 7.0 at 4°C, and centrifuged at 40000g for 1 h and the supernatant fraction was used for the assay of putrescine-dependent *S*-adenosyl-L-methionine decarboxylase as described by Pegg & Williams-Ashman (1969). The assay medium contained 100 μ mol of potassium phosphate buffer, pH 7.0, 2 μ mol of putrescine, 0.2 μ mol of *S*-adenosyl-L-[carboxy- 14 C]methionine (6.95 d.p.m./ μ mol) and up to 0.8 ml of the tissue extract containing up to 15 mg of protein. Results were expressed as nmol of 14 CO $_2$ released/mg of protein in a 30 min incubation at 37°C.

Results and Discussion

The greatest amount of methylglyoxal bis(guanylhydrazone) that could be administered as a single dose to the rats used in this study, without leading to death within 24 h, was 80 mg/kg body wt., which is in good agreement with other published values (Mihich, 1963). None of the rats used in the experiments was killed by this dose of the inhibitor, but all rats given 120 mg/kg body wt. died within 5 h. At 30 min after the administration of 80 mg/kg body wt. of methylglyoxal bis(guanylhydrazone), the activity of kidney putrescine-dependent *S*-adenosyl-L-methionine decarboxylase measured *in vitro* was less than 5% of that of control rats, and remained at this value for a further 6 h (Fig. 1). It then increased gradually, reaching 50% of the control value 12 h after the administration of the inhibitor, and was slightly greater than control values (115%) by 20 h. In these experiments the final assay conditions represented a 4-fold dilution of the kidney tissue, and the inhibition of *S*-adenosyl-L-methionine decarboxylase *in vivo* may therefore be greater than that obtained *in vitro*.

As shown in Fig. 2, methylglyoxal bis(guanylhydrazone) was a very potent inhibitor when added directly to the assay medium for kidney *S*-adenosyl-L-methionine decarboxylase, producing 50% inhibition of the release of CO $_2$ when present at a concentration of 0.25 μ M. This result confirms the report of Williams-Ashman & Schenone (1972), but the amount of inhibitor required to produce 50% inhibition of the kidney enzyme activity was significantly less than 1.25 μ M, which was needed to produce 50% inhibition of the activity of prostatic *S*-adenosyl-L-methionine decarboxylase in their experiments. It is therefore apparent that methylglyoxal bis(guanylhydrazone) is fairly rapidly removed from the kidney either by excretion or by metabolism, although the possibility that increased synthesis of the enzyme also takes place and contributes to the rise in activity between 6 and 20 h after the inhibitor was given (Fig. 1) cannot be ruled out.

Table 1 shows the effect of administration of methylglyoxal bis(guanylhydrazone) (80 mg/kg body wt.) on the polyamine content of liver and kidney and also shows the radioactivity present in each of the

Fig. 1. Activity of putrescine-dependent *S*-adenosyl-L-methionine decarboxylase from kidneys of rats treated with methylglyoxal bis(guanylhydrazone)

Decarboxylation of *S*-adenosyl-L-methionine catalysed by kidney extracts prepared from rats injected with 80 mg of methylglyoxal bis(guanylhydrazone)/kg body wt. at the time before death shown on the Figure was measured as described in the text. Supernatant (0.8 ml) from the kidney homogenates was used in each assay.

polyamines after the administration of [1,4- 14 C $_2$]-putrescine 4 h before death. Results obtained for the control animals are in reasonable agreement with other published data (Jänne *et al.*, 1964; Jänne, 1967; Russell *et al.*, 1970). In rats killed 8 h after receiving the inhibitor, incorporation of labelled putrescine into spermidine was inhibited by more than 90% in both liver and kidney. There was also a significant increase in the putrescine content of these tissues, but no significant fall in that of spermidine. These results provide good evidence that methylglyoxal bis(guanylhydrazone) does enter the cell and prevents spermidine synthesis at a step after the formation of putrescine. Rats killed 20 h after receiving the inhibitor showed a clear increase in the tissue putrescine content, which was increased 3-fold in liver and 2-3-fold in kidney, but there was only a slight fall in spermidine content. The incorporation of labelled putrescine into spermidine during the

even after the complete inhibition of spermidine formation, since the published value for the half-life of spermidine in liver is 4 days (Russell *et al.*, 1970). Since the dose of inhibitor used in these experiments was the maximum that could be tolerated by the rats as a single dose within a 24 h period, it is unlikely that methylglyoxal bis(guanyldihydrazone) can be used in the intact animal to obtain information on the role of spermidine in normal tissues. It may be possible to administer repeated smaller doses, which could still prevent spermidine synthesis over a longer period of time, or to use the inhibitor to study the function of the increased synthesis of spermidine that occurs rapidly after stimuli such as partial hepatectomy (Dykstra & Herbst, 1965; Raina *et al.*, 1966; Jänne, 1967), but studies of the effects of methylglyoxal bis(guanyldihydrazone) on the growth of cells in cultures which are unable to lower the concentration of the inhibitor by excretion or metabolism may be more rewarding.

The experiments described above, showing that methylglyoxal bis(guanyldihydrazone) can completely prevent the synthesis of spermidine from putrescine in rat liver and kidney, provide strong evidence that the putrescine-activated *S*-adenosyl-L-methionine decarboxylase that is inhibited by this compound *in vitro* is essential for spermidine synthesis in these tissues. The accumulation of putrescine in liver and kidney when spermidine formation is prevented in this manner suggests that spermidine synthesis is a major pathway for the metabolism of putrescine in these tissues. Although putrescine administered to rats by intraperitoneal injection is converted into polyamines to only a very small extent and a much greater amount is converted into CO₂ and other unidentified compounds (Jänne, 1967) there is probably a major difference between putrescine formed within the tissue and exogenous putrescine in this respect. The total dose of [1,4-¹⁴C₂]putrescine given in the present experiments to follow spermidine formation *in vivo* was 0.56 μmol, but measurement of the total radioactivity present in the polyamines of liver and kidney showed that only a small amount of this material (less than 4%) was present in the tissue polyamines, in agreement with the results of Jänne (1967). The increase in the putrescine concentration produced by the inhibitor must therefore have been caused by the continued formation of putrescine by ornithine decarboxylase (Pegg, 1970) while spermidine synthesis was inhibited.

Methylglyoxal bis(guanyldihydrazone) could not have decreased the labelling of spermidine by decreasing the uptake of labelled putrescine by the tissues, since the total radioactivity present in putrescine, spermidine and spermine was not diminished in animals treated with the inhibitor. In fact, in animals given the inhibitor 16 h before the labelled putrescine, the uptake of labelled putrescine

was markedly greater than in controls in both liver and kidney. The explanation for this finding requires further studies of the metabolism of putrescine administered by intraperitoneal injection.

Finally, although some radioactivity was present in spermine after the administration of labelled putrescine 4 h before death, the amount of incorporation was small, and it is not possible from the results to determine the extent to which methylglyoxal bis(guanyldihydrazone) inhibited the incorporation of spermidine into spermine. Conversion of spermidine into spermine appeared to occur to a greater extent in kidney than in liver, in agreement with the finding that kidney contains twice as much spermine as spermidine whereas in liver the amount of spermidine was greater than that of spermine (Table 1).

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