METHYLGLYOXAL-BIS (GUANYLHYDRAZONE) INHIBITS THE ACCUMULATION OF KIDNEY PROTEINS DURING COMPENSATORY RENAL HYPERTROPHY*

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Summary: Protein and RNA accretion occurs in the kidney following unilateral nephrectomy (UNI). Growth is essentially hypertrophic since virtually no change in the DNA content occurs. The protein accretion results from decreased protein catabolism. Methylglyoxal-bis (guanylhydrazone) [MGBG] inhibits the protein and RNA accretion in the mouse kidney following UNI. MGBG inhibits the activity of S-adenosyl-L-methionine decarboxylase and blocks the formation of spermidine and spermine. MGBG does not alter ^{3}H-leucine incorporation into kidney proteins in UNI mice. The MGBG treated UNI mice have a higher rate of kidney protein catabolism than the UNI mice. These results strongly suggest that spermidine and spermine are involved in the regulation of protein catabolism and that increased levels of these polyamines are necessary to decrease protein catabolism of kidney proteins after growth is stimulated by UNI.

Compensatory renal hypertrophy induced by unilateral nephrectomy (UNI) is marked after 48 hr by a 10-20% increase in protein content per cell. Following UNI no major increase in protein synthesis occurs and the decrease in protein degradation is responsible for protein accretion (1). Increased renal concentrations of putrescine, spermidine, and spermine seem to precede or parallel the early increase in protein accumulation (2,3,4). Methylglyoxal-bis (guanylhydrazone) [MGBG] inhibits the enzymic activity of S-adenosyl-L-methionine decarboxylase (5-9). Daily injections of MGBG results in a marked increase in the concentration of putrescine and a decrease in spermine and spermidine (10). This report shows the effects of MGBG on protein accretion and the mean half-life of kidney proteins after induction of renal hypertrophy by UNI.

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Materials and Methods

Male Swiss - Webster - NCI outbred mice (35-45 days, 30-34 g) were used in all experiments. Mice were maintained on Wayne chow and housed in quarters with a 12 hr light - 12 hr dark cycle. UNI and sham nephrectomy (sham) were performed as previously described (11). MGBG dihydrochloride was obtained from Microbiological Associates, through the courtesy of Dr. Harry B. Wood, Jr. National Cancer Institute, Bethesda, Maryland. MGBG was dissolved in 0.8% NaCl solution immediately prior to injections. Daily subcutaneous injections of MGBG or 0.8% NaCl were begun 2 or 3 days prior to UNI and continued until sacrifice. The dose of MGBG was always 50 µg/g of body weight.

The mean protein half-lives were determined as follows: One day prior to UNI mice were given 12.5 µC of ^{3}H-leucine (Schwarz/Mann, 46 C/mmole) or 10 µC guanido-^{14}C-L-arginine (Schwarz/Mann, 46 mC/mmole). Following UNI, mice were given L-leucine (400 µg/gm of body weight) or L-arginine (100 µg/gm body weight) to reduce reutilization of isotopically labeled amino acids. Protein concentration was assessed by the method of Lowry, et al. (12). DNA and RNA were extracted (13) and estimated respectively with the diphenylamine reaction (14) (calf-thymus DNA as the standard) and with the orcinol reaction (15) (yeast RNA as the standard).

Results

Table I shows the effects of MGBG on RNA and protein concentration in

TABLE I

Effects of MGBG on Kidney RNA/DNA and Protein/DNA

Time from Surgery to sacrifice	Treatment	RNA/DNA	Protein/DNA
24 hr	UNI/saline	1.54	88
24 hr	UNI/MGBG	1.19	80
24 hr	Sham/saline	1.20	78
24 hr	Sham/MGBG	1.22	82
48 hr	UNI/saline	1.74	96
48 hr	UNI/MGBG	1.18	78
48 hr	Sham/saline	1.21	79
48 hr	Sham/MGBG	1.23	75

Mice received daily subcutaneous injections of MGBG beginning 3 days prior to UNI. DNA, RNA and protein were determined as stated in methods section. Each value is an average from at least six mice.

the kidney. Accretion of RNA and protein is inhibited in the MGBG treated UNI mice, although the RNA/DNA and protein/DNA are not reduced in the MGBG treated sham mice. Apparently, the 4-5 day treatment with MGBG does not affect the RNA and protein content per kidney cell in the sham mice. Table II shows the results of MGBG on ^{3}H-leucine incorporation into kidney protein following UNI. The 4 hr labeling time ending 24 or 48 hr after UNI resulted in no difference in ^{3}H-leucine incorporation. The acid soluble radioactivity was the same in saline and MGBG mice. After an 18 hr labeling time ending 48 hr after UNI, the kidney proteins from the two groups had the same specific activity. The results in Fig. I show the mean half-lives of kidney proteins from saline and MGBG-treated UNI mice. The mean half-life of kidney proteins from MGBG treated UNI mice is decreased 23% using ^{3}H-leucine. Fig. 2 shows the degradation rate of proteins labelled with L-arginine (^{14}C-guanido). The difference in the mean half-lives is over 18%. The results in Fig. 1 and 2 are expressed as radioactivity in protein/DNA since during the time interval studied the DNA content is the same in the MGBG treated UNI mice and UNI mice. The mean half-life of liver protein from MGBG-treated UNI mice was unchanged from that of UNI mice.

Discussion

The results show that MGBG does not alter ^{3}H-leucine incorporation into protein, but does influence protein accretion by reducing the mean half-life of kidney proteins following induction of renal hypertrophy in UNI. An increase in protein degradation in the MGBG-treated UNI mice can account for the majority of the reduction in the protein/DNA (Table I). Although the biochemical function of the polyamines is not known, increasing evidence suggest their involvement in growth processes by affecting DNA, RNA, and protein metabolism (16-18). In general, the increased levels of the polyamines and the increased enzymic activity of ornithine decarboxylase

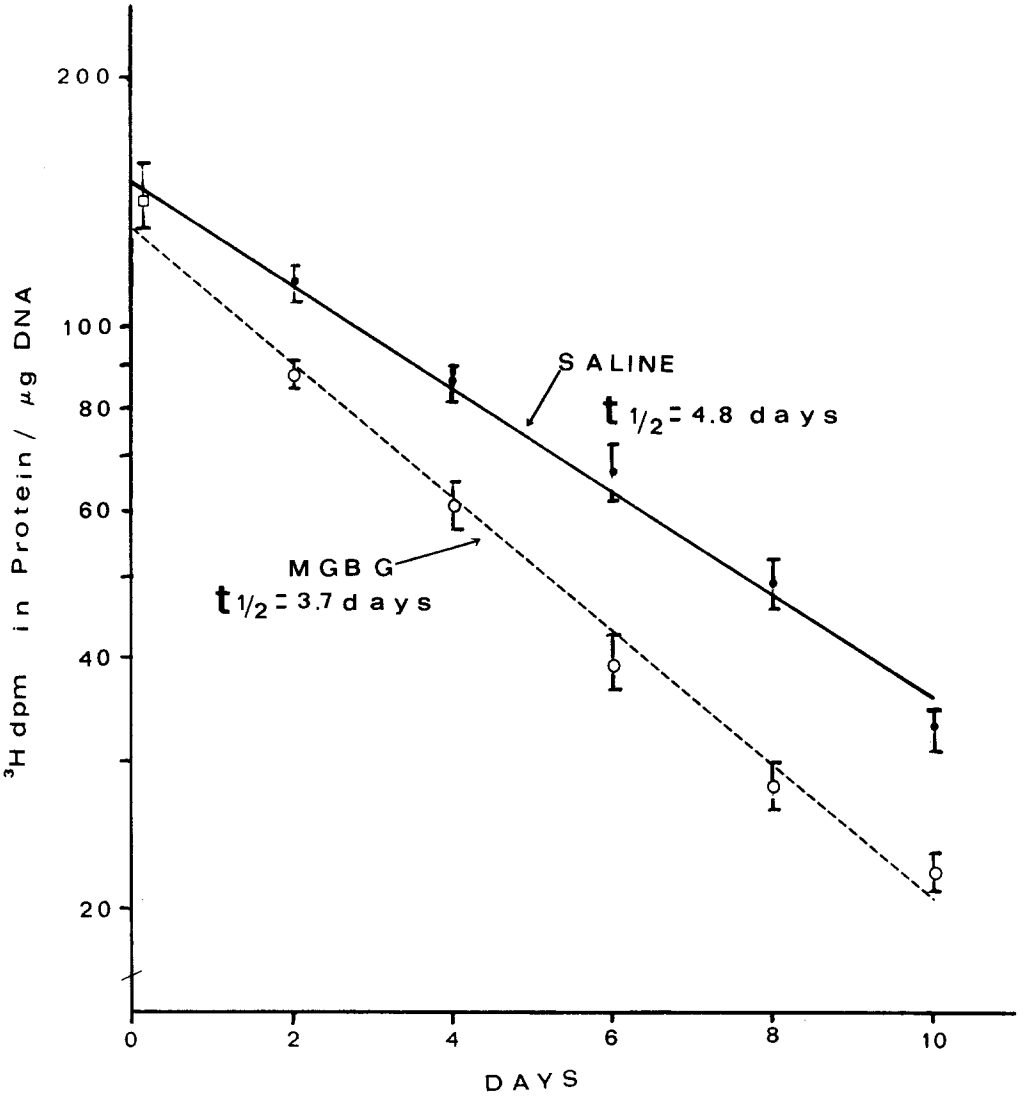

Fig. 1. Mice received daily subcutaneous injections of MGBG or saline beginning two days prior to UNI. One day before UNI, all mice received 12.5 µCi of ^{3}H-leucine by subcutaneous injection. After UNI, all mice received daily subcutaneous injections of unlabeled leucine (40 µg/gm body wt.). The kidneys removed at UNI were used to determine the zero time point. The slope of the lines were determined by the least squares method. Each point represents at least three animals. The ranges are shown on the vertical lines.

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seem to precede or parallel the increases in DNA synthesis and RNA and protein metabolism (16-18). Kay and Pegg (5) reported that MGBG inhibits protein and RNA synthesis in phytohaemagglutinin stimulated lymphocytes.

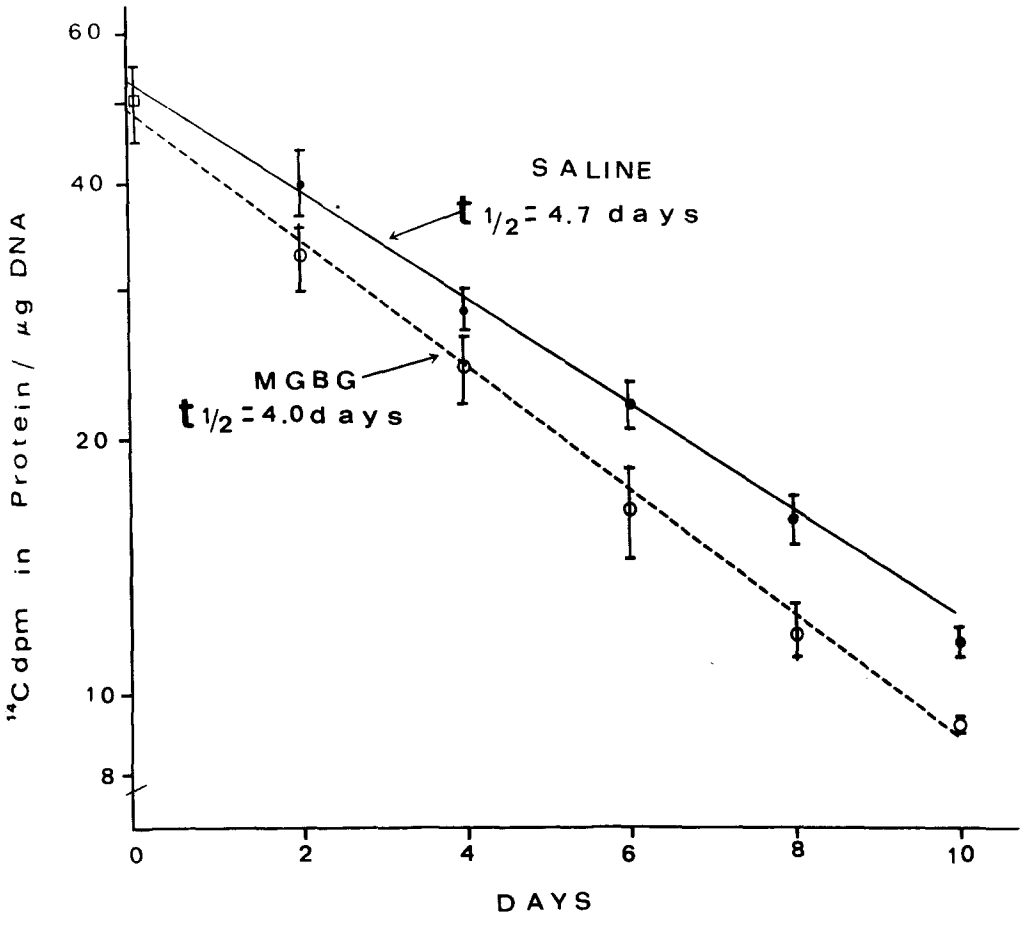

Fig. 2. Mice received daily subcutaneous injections of MGBG or saline beginning two days prior to UNI. One day before UNI, all mice received 10 µCi of arginine (guanido-^{14}C) by subcutaneous injection. After UNI, all mice received daily subcutaneous injections of unlabeled arginine (10 µg/gm body wt.). The kidneys removed at UNI were used to determine the zero time point. The slope of the lines were determined by the least squares method. Each point represents at least three animals. The ranges are shown on the vertical lines.

However, Fillingame and Morris (19), using concanavalin A transformed lymphocytes, reported no change in protein and RNA synthesis. The results in Table II suggest the MGBG has not altered protein synthesis, however, the drug does alter protein accumulation. The results in Fig. 1 and 2 indicate a significant decrease in the mean half-life of kidney protein from MGBG treated UNI mice. Although others (6,8,20) have reported an

TABLE II

Effects of MGBG on ^{3}H-Leucine Incorporation into Kidney Protein after uninephrectomy

Time from Surgery to Sacrifice	Treatment	Labeling Time	Protein (dpm/mg) mean ± S.D.
24 hr	saline	4 hr	2280 ± 190
24 hr	MGBG	4 hr	2360 ± 210
48 hr	saline	4 hr	2310 ± 185
48 hr	MGBG	4 hr	2190 ± 205
48 hr	saline	18 hr	1982 ± 170
48 hr	MGBG	18 hr	1965 ± 180

Mice received daily subcutaneous injections of MGBG or saline beginning 3 days prior to UNI. All mice received 12.5 μCi of ^{3}H-leucine by subcutaneous injection. The mean and standard deviations were determined from data from four mice.

increase in the half-life of S-adenosyl-L-methionine decarboxylase, this is the first report of the general effects of MGBG on protein catabolism during stimulated growth. Since a decrease in protein catabolism has been shown to cause the protein accretion during renal hypertrophy (1) and that MGBG blocks this accretion, I suggest that spermidine and spermine are involved in the mechanism(s) which control kidney protein catabolism during stimulated growth.

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