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STABILIZATION OF ORNITHINE DECARBOXYLASE IN MOUSE LIVER AND LUNG BY METHYLGLYOXAL BIS(CYCLOHEXYLAMIDINOHYDRAZONE)

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Abstract—The intraperitoneal injection of methylglyoxal bis(cyclohexylamidinohydrazone) (MGBC), an inhibitor of *S*-adenosylmethionine decarboxylase and spermidine synthase, markedly increased (7-fold of the basal level at 4 hr) ornithine decarboxylase (ODC) activity in normal mouse liver. ODC activity was also increased 2.5-fold over the basal level in mouse lung at 6 hr after the injection. The effect of MGBC on ODC activity occurred in a dose-dependent manner. Measurement of the apparent half-life of ODC induced in the liver and lung by MGBC treatment revealed a clear decrease in the decay rate of the enzyme in both the tissues. Activities of *S*-adenosylmethionine decarboxylase (AdoMetDC) and spermidine/spermine N^1-acetyltransferase (SAT) were not increased by the intraperitoneal injection of MGBC.

There was a large rise in putrescine and a fall in spermidine and spermine in the liver and lung except for brain within an 8 hr period in response to MGBC, suggesting that these changes resulted from the stabilization of ODC and inhibitions of AdoMetDC and spermidine synthase.

Ornithine decarboxylase (ODC; EC 4.1.1.17)* is a rate-limiting enzyme in the synthesis of polyamines, putrescine, spermidine and spermine. Many stimuli increase ODC activity, and the resultant increases in polyamine levels have been implicated in a variety of cellular actions responding to such stimuli [1–3]. Various compounds have also been shown to increase ODC activity [4–6]. Methylglyoxal bis(guanylhydrazone) (MGBG), a potent inhibitor of *S*-adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50), has been shown to stimulate ODC activity in animals [7, 8]. We have recently reported that MGBG increased the activities of ODC and spermidine/spermine N^1-acetyltransferase (SAT) in erythroid leukemia K562 cells [9]. In the previous paper [10] we reported a novel inhibitor of polyamine biosynthesis, methylglyoxal bis(cyclohexylamidinohydrazone) (MGBC), which exerted the inhibitory effects on AdoMetDC and spermidine synthase *in vitro*.

In the present study, we investigated *in vivo* effects of MGBC on polyamine synthetic enzymes in mouse tissues. ODC activity was increased in the liver and lung of mouse by a single intraperitoneal injection of the drug. The MGBC-stimulated increase of the enzyme activity was partially due to the decreased rate of degradation of the enzyme.

MATERIALS AND METHODS

Chemicals. MGBC, methylglyoxal bis(butylamidinohydrazone) (MGBB) and methylglyoxal bis(aminopropylamidinohydrazone) (MGBA) were synthesized according to the method previously published [11]. MGBG was purchased from Aldrich Chemical Co. (Milwaukee, WI). DL-[1-^{14}C]-Ornithine (48.8 mCi/mmol), [carboxy-^{14}C]-*S*-adenosyl-L-methionine (58.9 mCi/mmol) and [acetyl-1-^{14}C]-acetyl-CoA (59.8 mCi/mmol) were obtained from New England Nuclear (Boston, MA). All other chemicals were products of Nakarai Chemicals Ltd (Kyoto, Japan).

Animals and their treatment. Male DDY mice weighing 20–23 g, 6 weeks of age, were purchased from Shizuoka Agricultural Co-operative Association for Experimental Animals (Hamamatsu, Japan). The animals were fed mouse food MF (Oriental Yeast Co., Tokyo, Japan) and tap water *ad lib*. MGBC, MGBG, MGBB and MGBA were dissolved in 0.15 M NaCl before being injected intraperitoneally.

Preparation of enzyme extracts containing ODC and SAT. The animals were sacrified by dislocation. The liver, lung and brain were quickly removed and homogenized in 5 vol. of ice-cold buffer solution consisting of 50 mM Tris–HCl, pH 7.2, 0.1 mM EDTA, 10 mM dithiothreitol, and 40 μM pyridoxal-5′-phosphate. The homogenates were centrifuged at 100,000 *g* for 30 min at 2°. The resulting supernatants were used for the analyses of ODC and SAT activities. Protein was determined by the method of Bradford [12] using bovine serum albumin as a standard.

Preparation of enzyme extracts containing AdoMetDC. The livers were quickly removed and homogenized in 3 vol. of ice-cold buffer solution consisting of 10 mM Tris–HCl, pH 7.5, 2.5 mM putrescine, 1 mM dithiothreitol, and 0.1 mM EDTA.

* Abbreviations used: MGBC, methylglyoxal bis-(cyclohexylamidino-hydrazone); MGBG, methylglyoxal bis(guanylhydrazone); ODC, ornithine decarboxylase; AdeMetDC, *S*-adenosylmethionine decarboxylase; SAT, spermidine/spermine N^1-acetyltransferase.

The homogenates were centrifuged at 100,000 *g* for 30 min at 2°. The resulting supernatants were used for the analysis of AdoMetDC activity.

Assay for ODC. ODC activity was determined by measuring the release of $^{14}CO_2$ from DL-[^{14}C]-ornithine as described by Seely *et al.* [13]. The assay mixture contained 0.1 mM DL-[1-^{14}C]-ornithine (0.2 μCi), 20 μM pyridoxal-5'-phosphate, 5 mM dithiothreitol, 50 mM Tris–HCl, pH 7.2, and enzyme extract in a final volume of 0.2 ml.

Assay for AdoMetDC. AdoMetDC activity was determined by measuring the release of $^{14}CO_2$ from [carboxy-^{14}C]-*S*-adenosyl-L-methionine as described by Pegg and Williams-Ashman [14]. The assay mixture contained 10 mM putrescine, 2 mM AdoMet (0.1 μCi), 10 mM dithiothreitol, 10 mM Tris–HCl, pH 7.5, and enzyme extract in a final vol. of 0.4 ml.

Assay for SAT. SAT activity was determined by measuring the incorporation of the acetyl-1-^{14}C-group of acetyl-CoA into acetylspermidine as described by Matsui and Pegg [15]. The assay mixture contained 3 mM spermidine, 8 μM acetyl-[1-^{14}C]-acetyl-CoA, 50 mM Tris–HCl, pH 7.8, and enzyme extract in a final vol. of 0.2 ml.

Determination of polyamines. The liver, lung and brain were homogenized in 5 vol. of 0.4 N perchloric acid. The homogenates were centrifuged at 10,000 *g* for 20 min. The resulting supernatants were used for polyamine determination by HPLC (Shimazu LC-5A) as described previously [16].

RESULTS AND DISCUSSION

Figure 1 shows the time course of the change in hepatic ODC activity after intraperitoneal injections of MGBC or the related compounds at a dose of 60 mg/kg, respectively. The injection of MGBC markedly increased ODC activity, reaching a maximum (7-fold of the basal level) at 4 hr and returned to the basal level by 8 hr. Unlike MGBC, MGBG increased ODC activity more than 8 hr after the injection in agreement with the result of Karvonen [17].

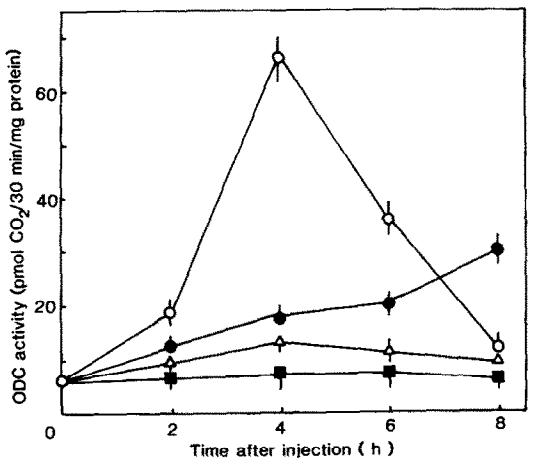

Fig. 1. Comparison of the increase in ODC activity after a single injection of MGBC and the related compounds in mouse liver. MGBC (○), MGBG (●), MGBB (△) and MGBA (■) (60 mg/kg, respectively) were injected intraperitoneally at time 0 and the mice were killed at the times shown. ODC activity of the liver was determined as described in the text. Each point and bar represents the mean value and SD, respectively, of three independent experiments.

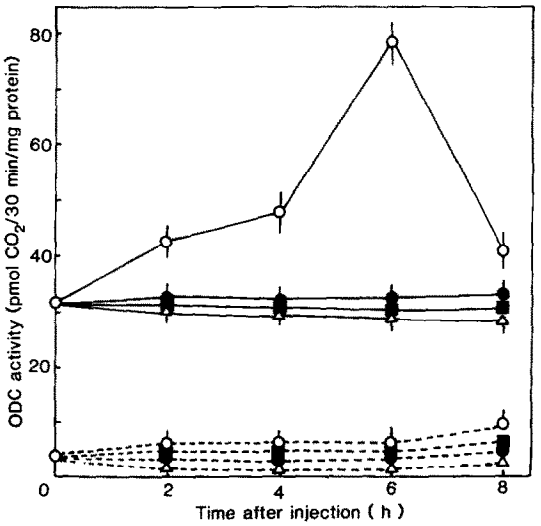

Fig. 2. Comparison of the increase in ODC activity after a single injection of MGBC and related compounds in mouse lung and brain. MGBC (○), MGBG (●), MGBB (△) and MGBA (■) (60 mg/kg, respectively) were injected intraperitoneally at time 0 and the mice were killed at the times shown. ODC activity of the lung (solid lines) or brain (dotted lines) was determined as described in the text. Each point and bar represents the mean value and SD, respectively, of three independent experiments.

As shown in Fig. 2, the intraperitoneal injection of MGBC also increased ODC activity in the mouse lung, reaching a maximum (2.5-fold of the basal level) at 6 hr, and returned to the basal level by 8 hr. Methylglyoxal hydrazone analogs other than MGBC did not increase ODC activity in the lung. On the other hand, the enzyme activity in the brain of the mouse was not increased by MGBC at all, which may be explained by MGBC not passing through the blood–brain barrier. The activity of SAT in these tissues was not increased by the intraperitoneal injections of these drugs (data not shown). The activity of AdoMetDC in the liver was not increased at 24 hr after the intraperitonal injections of these drugs other than MGBG (60 mg/kg, respectively). The injection of MGBG increased AdoMetDC activity of the liver by 15-fold at 24 hr after the injection as has been reported in another investigation [18].

Dose response effect of MGBC on ODC activity was examined at 5 hr after the injection of the drug. As shown in Table 1, MGBC was able to increase ODC activity at a dose of 20 mg/kg. The highest dose of MGBC (100 mg/kg) used in this study increased ODC activity to about 11-fold (for liver) and 3-fold (for lung) over the basal level. The increase of ODC activity by MGBC is likely to be a specific effect, since the drug even at the same or higher dose used in this study has been shown to be non-toxic to mice [10]. Thus it is unlikely that the

Table 1. Dose response effect of MGBC on ODC activity in mouse liver and lung

Treatment	ODC activity (pmol/30 min/mg protein)
(A) Liver	
Control	6.80 ± 0.46
MGBC (20 mg/kg)	12.51 ± 1.78
MGBC (60 mg/kg)	50.72 ± 5.25
MGBC (100 mg/kg)	72.53 ± 7.82
(B) Lung	
Control	29.85 ± 2.57
MGBC (20 mg/kg)	45.25 ± 5.11
MGBC (60 mg/kg)	68.38 ± 6.13
MGBC (100 mg/kg)	85.91 ± 7.89

Mice were injected intraperitoneally with MGBC at the dose indicated and killed 5 hr later. ODC activity was determined as described in the text. The values are the mean ± SD for three mice.

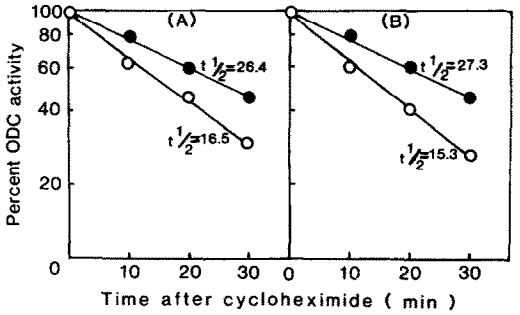

Fig. 3. Effect of MGBC on the half-life of ODC in normal mouse liver (A) and lung (B). MGBC injection (60 mg/kg) (●) was performed 5.5 hr before the death of the animals. Control mice (○) were given saline instead of MGBC solution. Cyclohexamide (20 mg/kg) was injected intraperitoneally at the times shown before the death of the animals.

increased ODC activity elicited by MGBC in mice is due to a compensatory response to possible hepatic and pulmonary toxic effects.

The increase of ODC activity seen after the injection of MGBC was almost completely inhibited by pretreatment of mice with either cycloheximide (20 mg/kg) or actinomycin D (2.5 mg/kg) (data not shown). The results indicate that there is probably not any activation of preformed ODC.

The half-life of ODC was determined in the liver and lung using cycloheximide after 5 hr of MGBC induction. As shown in Fig. 3, the half-life of ODC increased in mouse liver and lung by a single injection of MGBC was longer than that of the control. The finding suggested that there was the stabilization effect on the ODC enzyme protein in both tissues.

The effects of MGBC or related compounds on polyamine levels in the liver, lung and brain were examined at 8 hr after the injection of the drugs. As shown in Table 2, putrescine was increased by MGBC, and the spermidine and spermine contents were slightly reduced by the drug within an 8 hr period in the liver and lung except for brain, suggesting that these changes resulted from the stabilization of ODC (Fig. 3) and inhibition of AdoMetDC and spermidine synthase [10]. The lack of effects of compounds other than MGBC on ODC reflected their inability to alter putrescine levels.

Table 2. Effect of MGBC and related compounds on polyamine levels in mouse liver, lung and brain

Treatment (dose)	Putrescine	Spermidine (nmol/g wet weight)	Spermine
(A) Liver			
Control	5.0 (100)	504.3 (100)	662.3 (100)
MGBC (60 mg/kg)	38.3 (766)	465.8 (92)	603.8 (91)
MGBG (60 mg/kg)	7.8 (156)	472.5 (94)	612.9 (93)
MGBB (60 mg/kg)	5.1 (102)	479.8 (95)	632.5 (96)
MGBA (60 mg/kg)	4.9 (98)	497.2 (99)	641.5 (97)
(B) Lung			
Control	7.3 (100)	319.3 (100)	187.3 (100)
MGBC (60 mg/kg)	27.5 (377)	288.5 (90)	165.8 (88)
MGBG (60 mg/kg)	10.8 (148)	292.5 (92)	171.9 (92)
MGBB (60 mg/kg)	6.8 (93)	299.6 (94)	177.3 (95)
MGBA (60 mg/kg)	7.0 (96)	313.3 (98)	179.8 (96)
(C) Brain			
Control	0.5 (100)	235.3 (100)	115.3 (100)
MGBC (60 mg/kg)	0.4 (80)	228.5 (97)	118.5 (103)
MGBG (60 mg/kg)	0.5 (100)	238.5 (101)	110.6 (96)
MGBB (60 mg/kg)	0.5 (100)	227.8 (97)	116.2 (101)
MGBA (60 mg/kg)	0.4 (80)	229.8 (98)	118.7 (103)

Mice were injected intraperitoneally with MGBC or related compounds. Control mice were given saline. The animals were killed 8 hr after the injection. Polyamine contents were determined as described in the text. Each value is the mean of dulplicate experiments. The per cent of the control (without treatment) is shown in parentheses.

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