Mem. Fac. Agr. Kagoshima Univ., **23**, 93-98, 1987

Effects of Actinomycin D and Cycloheximide on Histidine Decarboxylase Activities and Histamine Contents in the Spleen and Skin of Magnesium-Deficient Rats

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Received for Publication September 10, 1986

Introduction

It has been reported that the activity of histidine decarboxylase (HDC) in various tissues is induced to increase by many kinds of stimulants[6,14-17,19]. In the previous papers[12,13], we reported that the HDC activity of the spleen appeared to rise until the 8th day of magnesium (Mg)-deficiency, and then the rise of histamine content occurred, and the HDC activity of the skin increased also, but there was no rise in histamine content.

In order to see whether the increased HDC activity in both tissues is due to increased synthesis of the HDC enzyme or to activation of HDC or to any other mechanisms, examinations were carried out on to the effects of actinomycin D, an inhibitor of RNA synthesis[7], and cycloheximide, an inhibitor of protein synthesis[5], upon the HDC activities and histamine contents in the spleen and skin of Mg-deficient rats.

Materials and Methods

Animals and diet: Wistar rats of both sexes, weighing about 60 g body weight, were used. Before the beginning of experiments, the rats were fed with a powdered diet containing 0.07 % Mg (control diet) for 3 days. After that, the rats were fed for 8 days with a Mg-deficient diet (0.001 % Mg). Both diets were identical to each other in the contents of all the essential nutrients excepting Mg[12]. The respective diets and deionized water were provided ad libitum. The rats were housed in stainless-steel cages and kept at an ambient temperature 22-24°C with a controlled 12-hour light-dark cycle.

Preparation of samples and assay methods: Actinomycin D (200 or 400μg/kg., i. p.) or cycloheximide (1 or 2 mg/kg., i. p.) was administered on the 4th or 8th day after the feeding of Mg-deficient diet. The rats injected with these inhibitors were killed by heart puncture under light ether anesthesia at 3, 6 and 24 hr after injection. Actinomycin D and cycloheximide were obtained from Sigma Chemical Company. The skin of the back and the spleen were each removed, weighed and immediately homogenized in 10 volumes of 0.05 M sodium-potassium phosphate buffer (pH 7.0) for HDC assay or 0.4 N perchloric acid for histamine assay. The homogenate with 0.05 M sodium-potassium phosphate buffer was centrifuged for 30 min at 10,000×g, and the supernatant fluid was used for HDC assay.

These procedures were carried out at 4°C. The HDC (EC 4. 1. 1. 22) activity was assayed by the method of Kobayashi[10]. L-[carboxy-^{14}C] histidine with a specific activity of 51 mCi/mmol was used in HDC assay. The radioactive histidine was obtained from Amersham International Ltd. The protein contents of the samples were determined by the method of Lowry *et al.*[11]. The enzyme activity was calculated and expressed on a tissue protein basis. Histamine in the homogenate with 0.4 N perchloric acid was determined by the method of Shore *et al.*[18].

Statistical evaluations: The mean values of the results are presented in figures and table with standard deviations. Differences between means were tested for statistical significance by Student's *t*-test. The significance was established when the probability level was equal to, or less than 5 %.

Results

1. Effects of actinomycin D and cycloheximide on HDC activities in the skin and spleen of Mg-deficient rats

Fig. 1 shows the effects of actinomycin D and cycloheximide on HDC activity in the spleen. The activity of HDC in the spleen started to increase on the 4th day after the feeding of Mg-deficient diet, and then increased gradually until the 8th day. These results were similar to the previous one[13]. On the 4th day, the administration of actinomycin D (200 μg/kg., i. p.) had no significant effect on the splenic HDC activity, but cycloheximide (1 mg /kg., i. p.) decreased the splenic HDC activity significantly. On the 8th day, actinomycin D

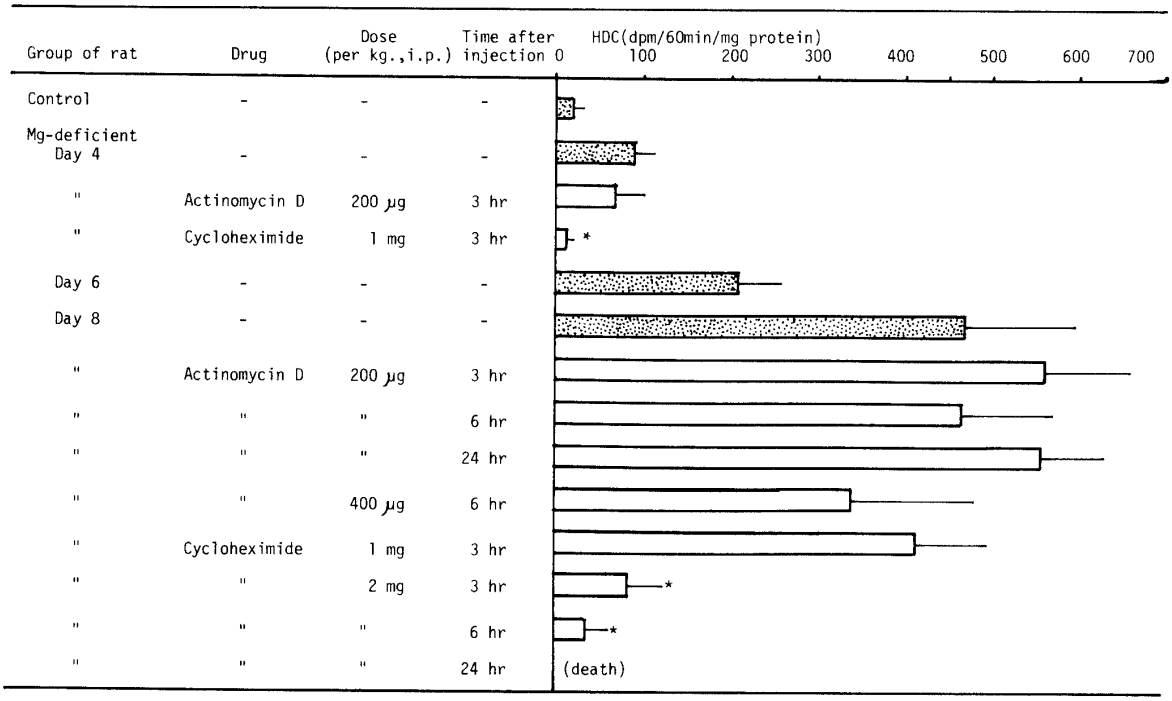

Fig. 1. Effects of actinomycin D and cycloheximide on histidine decarboxylase (HDC) activity in spleen of Mg-deficient rats. Each bar is a mean ± S. D. of five to ten rats. Asterisks indicate statistically significant differences between non-treated and drug-treated rats ($P<0.05$).

had no significant effect on the splenic HDC activity even though 400 μg/kg was administered, but cycloheximide decreased it markedly.

Fig. 2 shows the effects of actinomycin D and cycloheximide on HDC activity in the skin. The HDC activity in the skin was very high on the 4th day, but lower on the 6th day, then increased again on the 8th day of Mg-deficiency. The appearance-time-course of the HDC activity was similar to the results of the previous report[13]. On the 4th day, the administration of actinomycin D (200 μg/kg., i. p.) had no significant effect on the HDC activity of the skin, but a tendency to enhance the HDC activity was observed. Cycloheximide (2 mg/kg., i. p.) showed no significant effect on the HDC activity of the skin on the 4th day. On the 8th day, actinomycin D (200 or 400 μg/kg., i. p.) had no significant effect on the HDC activity of the skin, but cycloheximide (2mg/kg., i. p.) significantly decreased the HDC activity of the skin. Twenty-four hours after the injection of cycloheximide (2 mg/kg., i. p.) all the rats died.

2. Effects of actinomycin D and cycloheximide on histamine contents in the skin and spleen of Mg-deficient rats

Table 1 shows the effects of actinomycin D and cycloheximide on histamine contents in the skin and spleen. On the 8th day, the histamine content in the spleen increased markedly, but not in the skin. These data were similar to those of the previous report[12]. On the 8th day of Mg-deficiency, 24 hr after the administration of actinomycin D (200 μg/kg., i. p.) or cycloheximide (1 mg/kg., i. p.), the histamine contents in the skin and spleen were estimated. Actinomycin D had no significant effect on histamine contents in both tissues. Cycloheximide decreased the histamine content in the spleen to about 50% of non-administered Mg-deficient

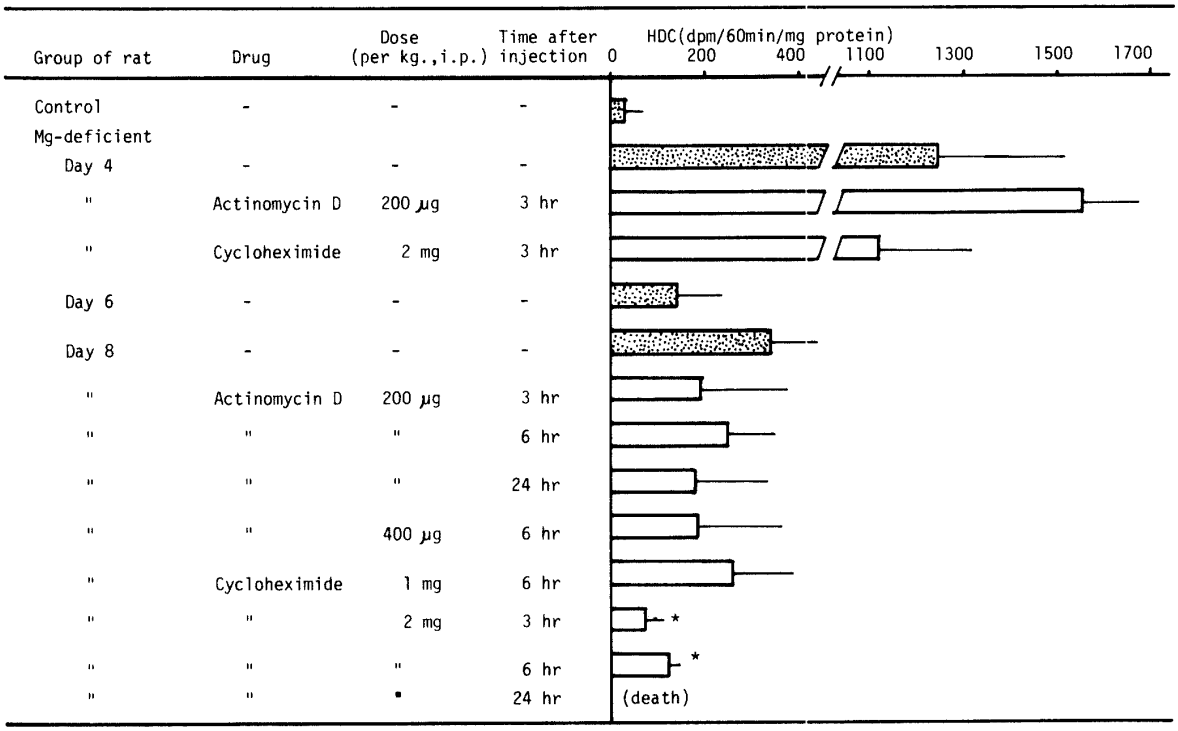

Fig. 2. Effects of actinomycin D and cycloheximide on histidine decarboxylase (HDC) activity in skin of Mg-deficient rats. Each bar is a mean ± S. D. of five to ten rats. Asterisks indicate statistically significant differences between non-treated and drug-treated rats ($P<0.05$).

Table 1. Effects of actinomycin D and cycloheximide on histamine contents in spleen and skin of Mg-deficient rats

Group of rat	Drug	Dose (per kg., i. p.)	Time after injection	Histamine (μg/g wet wt.) Spleen	Skin
Control	–	–	–	0.63 ± 0.23	28.83 ± 2.84
Mg-deficient					
Day 8	–	–	–	31.50 ± 11.70	29.86 ± 5.10
〃	Actinomycin D	200 μg	24 hr	35.24 ± 6.28	28.56 ± 2.53
〃	Cycloheximide	1 mg	24 hr	15.40 ± 4.21*	28.75 ± 2.54

Values are means ± S. D. of five to ten rats. Asterisk indicates statistically significant difference between non-treated and drug-treated rats ($P<0.05$).

spleen, but in the skin it was not significant.

Discussion

The increased HDC activity in the spleen during Mg-deficiency was depressed significantly after the administration of cycloheximide. The increased HDC activity in the skin was also depressed significantly after the administration of cycloheximide on the 8th day, but not on the 4th day of Mg-deficiency. These data suggest that the newly synthesized protein is necessary to increase the HDC activity in the spleen during Mg-deficiency, and in the skin on the 8th day, but not on the 4th day of Mg-deficiency.

The increased HDC activity in the spleen and skin was not depressed by the administration of actinomycin D. These results might show that newly synthesized protein involved in the increase of HDC activity is not blocked by the inhibition of RNA synthesis concerned with the production of HDC, or as suggested by Schayer and Reilly[15], long-lived RNA molecules might direct the protein synthesis.

On the 4th day of Mg-deficiency, the increased HDC activity in the skin was not depressed by the administration of cycloheximide. The data suggest that the increased HDC activity in the skin on the 4th day of Mg-deficiency may depend on the activation of HDC, and not on the newly synthesized protein.

It is well known that the rat skin has large numbers of mast cells which contain HDC and histamine, and that the degranulation of mast cells during early period of Mg-deficiency is observed[1-4]. We also confirmed these phenomena in the skin on the 4th day of Mg-deficiency[8]. Schayer *et al.*[17] reported that the repeated injection of compound 48/80, a histamine liberator, to rat resulted in a marked increase in HDC activity of the skin, but other histamine liberator, dextran, did not, and they suggested that the treatment with compound 48/80 stimulates or leads to the formation of new mast cells in rat skin, which have strong HDC activity. It has been suggested that the rapid increase in HDC activity is associated with "inducible HDC" in non-mast cells and that histamine synthesized by the inducible HDC is not stored in cells but is released immediately[9,14]. The histamine content of the skin did not increase during Mg-deficiency[12]. The increase in HDC activity of the skin on the 4th day of Mg-deficiency may be due to activation of HDC in new mast cells

and non-mast cells.

As reported previously[12,13], the increased histamine content of the spleen during Mg-deficiency was correlated with the increase in HDC activity. So the high splenic histamine content might have reflected the increased histamine-binding capacity in addition to the high HDC activity. We found that on the 8th day of Mg-deficiency there was the appearance of a lot of basophilic cells in the spleen[8] which were known to be capable of storing the histamine. It is possible to assume that cycloheximide depressed the new synthesis of protein which was related to histamine-binding capacity in basophilic cells. It is necessary to examine what kind of cells should contribute to the increasing in HDC activity and the binding of histamine during Mg-deficiency.

Summary

The feeding of Mg-deficient diet (0.001 % Mg) on young rats induced the increased histamine content with the increased HDC activity in the spleen, but in the skin the increased HDC activity was observed without the increasing of histamine content. The increased HDC activity in the spleen was depressed by the administration of cycloheximide on the 4th and 8th days of Mg-deficiency. In the skin the increased HDC activity was depressed by cycloheximide on the 8th day, but not on the 4th day, of Mg-deficiency. The increased HDC activity in the both tissues was not depressed by the administration of actinomycin D. The histamine content increased with an increased HDC activity in the spleen and was depressed with cycloheximide and not with actinomycin D. The histamine content in the skin did not increase during Mg-deficiency, and was not influenced by the administration of cycloheximide or actinomycin D. These results suggest that the newly synthesized protein is to be involved in the increasings of HDC activity and histamine content of the spleen during Mg-deficiency and that the mechanisms in the skin may be more complex than those in the spleen.

References

1) Bélanger, L. F., Van Erkel, G. A. and Jakerow, A.: Behavior of the dermal mast cells in magnesium-deficient rats. *Science*, **126**, 29–30 (1957)
2) Bois, P.: Effect of magnesium deficiency on mast cells and urinary histamine in rats. *Br. J. Exptl. Pathol.*, **44**, 151–155 (1963)
3) Bois, P., Byrne, E. H. and Bélanger, L. F.: Effect of magnesium deficiency on the regeneration of mast cells after treatment with 48/80 in rats. *Can. J. Biochem. Physiol.*, **38**, 585–589 (1960)
4) Bois, P., Gascon, A. and Beaulnes, A.: Histamine liberating effect of magnesium deficiency in the rat. *Nature*, **197**, 501–502 (1963)
5) Colombo, B., Felicetti, L. and Buglionic, C.: Inhibition of protein synthesis by cycloheximide in rabbit reticulocytes. *Biochem. Biophys. Res. Commun.*, **18**, 389–395 (1965)
6) Endo, Y.: Simultaneous induction of histidine and ornithine decarboxylases and changes in their product amines following the injection of *Escherichia coli* lipopolysaccharide into mice. *Biochem. Pharmacol.*, **31**, 1643–1647 (1982)
7) Garren, L. D., Howell, R. R., Tomkins, G. M. and Crocco, R. M.: A paradoxical effect of

actinomycin D : The mechanism of regulation of enzyme synthesis by hydrocortisone. *Proc. Natl. Acad. Sci. U. S. A.*, **52**, 1121-1129 (1964)

8) Ishiguro, S., Nishio, A., Miyao, N., Morikawa, Y., Takeno, K. and Yanagiya, I. : Studies on histamine containing cells in the spleen of magnesium deficient rats. *Folia pharmacol. japon*, **83**, 15P (1984)

9) Kahlson, G. and Rosengren, E. : New approaches to the physiology of histamine. *Physiol. Rev.*, **48**, 155–196 (1968)

10) Kobayashi, Y. : Determination of histidine decarboxylase activity liquid scintillation counting of $^{14}CO_2$. *Anal. Biochem.*, **5**, 284–290 (1963)

11) Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. : Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275 (1951)

12) Nishio, A., Ishiguro, S., Ikegaki, I. and Miyao, N. : Toxicological and pharmacological studies on the magnesium deficiency in rats. I. Histamine contents in some tissues of magnesium deficient rats. *Jpn. J. Vet. Sci.*, **44**, 653–659 (1982)

13) Nishio, A., Ishiguro, S. and Miyao, N. : Toxicological and pharmacological studies on the magnesium deficiency in rats : Histamine-metabolizing enzymes in some tissues of magnesium-deficient rats. *Jpn. J. Vet. Sci.*, **45**, 699–705 (1983)

14) Schayer, R. W. : Enzymatic formation of histamine from histidine, Rocha e Silva, (ed.), *Handbook of Experimental Pharmacology*, vol. XVIII, part 1, p. 688–725, Springer, Berlin (1966)

15) Schayer, R. W. and Reilly, M. A. : Suppresion of inflammation and histidine decarboxylase by protein synthesis inhibitors. *Am. J. Physiol.*, **215**, 472–476 (1968)

16) Schayer, R. W. and Reilly, M. A. : Studies on the mechanism of activation and deactivation of histidine decarboxylase. *Eur. J. Pharmacol.*, **20**, 271–280 (1972)

17) Schayer, R. W., Rothschild, Z. and Bizony, P. : Increase in histidine decarboxylase activity of rat skin following treatment with compound 48/80. *Am. J. Physiol.*, **196**, 295–298 (1959)

18) Shore, P. A., Burkhalter, A. and Cohn, V. H. : A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exptl. Ther.*, **127**, 182–186 (1959)

19) Watanabe, T., Taguchi, Y., Sasaki, K., Tsuyama, K. and Kitamura, Y. : Increase in histidine decarboxylase activity in mouse skin after application of the tumor promoter tetradecanoylphorbol acetate. *Biochem. Biophys. Res. Commun.*, **100**, 427–432 (1981)