Histamine Content and Histidine Decarboxylase Activity in the Spleen of the Magnesium-Deficient Rat: Comparison with the Skin and Peritoneal Mast Cells

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Abstract—In young rats, some effects of magnesium (Mg) depletion in the diet on histamine content and histidine decarboxylase (HDC) activity in spleen were studied in comparison with those in the skin and peritoneal mast cells. In the case of the young rats fed a Mg-deficient diet (0.001% Mg), the splenic histamine contents increased to levels about 1.3, 2.8 and 23 times as high as those in the rats fed a control diet (0.07% Mg) on the 4th, 6th and 8th day, respectively; histamine contents in the peritoneal mast cells also increased to about 1.2 and 1.8 times the control levels on the 6th and 8th day, respectively; no change was observed in histamine contents in the skin during 8 days of Mg depletion. HDC activities in the spleen of Mg-deficient rats on the 4th, 6th and 8th day increased to levels about 5.5, 15.5 and 35 times as high as the respective control values; the activities in the skin increased to about 37, 7 and 10 times the control values on the 4th, 6th and 8th day, respectively; while in the peritoneal mast cells, the activities increased to about 1.2 and 2.2 times the control values on the 6th and 8th day, respectively. On the 8th day of the Mg deficiency, some studies were made on the effects of histamine releasers on histamine contents in the spleen and peritoneal mast cells. Compound 48/80 (0.5 µg/ml) or polymyxin B (5 µg/ml) induced a release of histamine from the peritoneal mast cells, but not from the spleen cells isolated from the Mg-deficient rat in vitro. These results suggest that the effects of Mg deficiency on the spleen cells and on the mast cells were different.

Recently, it was observed that there was a close relationship between Mg deficiency and the increase in HDC activities in the spleen, skin and some other tissues of young rats (1). In the spleen, the histamine content increased with an elevated HDC activity, but no increase was found in the histamine content of the skin in spite of an elevation in HDC activity (2).

It has been well known that the histamine in the skin is stored in the tissue mast cells (3). Previously, we reported that the number of mast cells in the skin and spleen showed no significant changes on the 8th day of Mg depletion, while at that time, the histamine content and HDC activity in the spleen increased (1, 2). Therefore, it appears that no direct relationship exists between the Mg deficiency-induced increase in splenic histamine content which is associated with the elevation of HDC activity and the number of mast cells in this organ.

Differences in the change in histamine content and HDC activity between the spleen and the skin of the Mg-deficient rat promoted the investigation of the mechanisms involved in synthesis, storage and release of histamine in the spleen cells and mast cells.

In this study, peritoneal mast cells were used as a model of skin mast cells to obtain more information about the synthesis, storage
and release of histamine in the Mg-deficient rat. A preliminary report of the results was presented previously (4).

Materials and Methods

Materials: L-[carboxyl-14C]histidine with a specific activity of 55 mCi/mmol (Amer- sham International Ltd.) was used in the HDC (EC 4.1.1.22) assay. Compound 48/80 and polymyxin B sulfate were obtained from Sigma Chemical Co. All other chemicals used were standard chemical products.

Animals and diet: Wistar rats of both sexes, weighing about 60 g, were randomly assigned into two groups that were fed for 8 days on a powdered diet containing 0.07% (control) or 0.001% Mg (deficient). Both diets were identical to each other in the contents of all the essential nutrients except Mg. The composition of the diet was the same as that reported previously (2). The respective diets and deionized water were provided ad libitum. The rats were housed in stainless steel cages and kept at an ambient temperature of 22–24°C and under a 12-hr light-dark cycle.

Preparation of samples and assay methods: Two groups consisting of six or more rats from the Mg-deficient and control groups were killed by decapitation at 2-day intervals up to the 8th day. The skin of the back, spleen and peritoneal mast cells were collected. The peritoneal mast cells were obtained by washing the peritoneal cavity with phosphate buffer in accordance with the method of Sugiyama (5). Spleen cells were isolated by the following method: Removed spleens were shredded by scissors and strained through a stainless steel sieve in phosphate buffer, and further disruption was achieved by gentle aspiration with a Pasteur pipette, followed by filtration through gauze. The single cells thus obtained were washed with phosphate buffer by centrifugation for 5 min at 150 g. Histamine was determined spectrofluorometrically by the method of Shore et al. (6). All the data were expressed in terms of μg histamine base contained per gram of tissue or 10⁶ mast cells. HDC activity in tissue or mast cells was assayed by the method of Kobayashi (7). The calculation and expression of the activity was carried out based on the amount of tissue protein (dpm/mg) or mast cell number (dpm/10⁴ mast cells). The protein contents of samples were determined by the method of Lowry et al. (8).

Statistical evaluations: The mean values of the results are presented in tables and figures. Differences between the 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. Histamine contents in the spleen, skin and peritoneal mast cells in Mg-deficient rat

Histamine contents were determined in the tissues of spleen, skin and peritoneal mast cells on the 2nd, 4th, 6th and 8th day after feeding on the Mg-deficient diet. As shown in Fig. 1, histamine content increased to about 23 times the control level in the spleen on the 8th day of Mg depletion. No increase was observed in the skin. On the other hand, histamine content of the peritoneal mast cells increased to about 2 times the control level on the 8th day of Mg depletion. These results suggest that the effects of Mg depletion on the histamine contents of the different tissues are mutually different.

2. Histamine release by compound 48/80 or polymyxin B

Fig. 1. Effect of magnesium deficiency on histamine content in the skin, spleen and peritoneal mast cells. Each bar represents the mean of six to ten rats. Asterisks indicate statistically significant differences between the Mg-deficient and control rats (P<0.05).
i) In vivo experiments: Two histamine releasers, compound 48/80 (9) and polymyxin B. (10–12), known to release histamine from mast cells, were used to examine the mode of the storage of histamine in the spleen. An hour after the subcutaneous injection of these releasers (Table 1), estimations were carried out on histamine contents in the spleen and skin of the control and the Mg-deficient rats on the 8th day of Mg depletion. Compound 48/80 (200 µg/100 g, b.w.) and polymyxin B (500 µg/100 g, b.w.) caused histamine release from the skin of both control and Mg-deficient rats, and the extent of the release in the Mg-deficient rats was less than that in the controls.

Splenic histamine content of the control rats increased after the administration of both of these releasers, while that of the Mg-deficient rats was decreased by the same releasers.

ii) In vitro experiments: In vitro experiments on isolated spleen cells and peritoneal mast cells from both the control and the Mg-deficient rats are shown in Tables 2 and 3. Compound 48/80 or polymyxin B released about 80–90% of the mast cell histamine in the control rats, while only about 25–50% of the mast cell histamine was released by these releasers in Mg-deficient rats on the 8th day of Mg depletion.

Histamine contents of the spleen cells isolated from Mg-deficient rats were about 10 times as high as those of the control rats. The spleen cells isolated from both control and Mg-deficient rats show no sensitivity to these releasers.

3. HDC activities in the spleen, skin and peritoneal mast cells in Mg-deficient rat
The HDC activities of the crude extracts

Table 1. Effects of compound 48/80 and polymyxin B on tissue histamine contents of the control and magnesium-deficient rats in vivo

|                | Non-treated                       | Treated with |             |
|----------------|-----------------------------------|--------------|-------------|
|                | Histamine content (µg/g, wet tissue) | Compound 48/80 | Polymyxin B |
| Control        |                                   |              |             |
| Skin           | 38.4±10.36                        | 53.4±20.1    | 60.8±36.0   |
| Spleen         | 1.03±0.68                         | 309.7±157.9  | 568.5±352.9 |
| Mg-deficient*  |                                   |              |             |
| Skin           | 40.3±9.20                         | 84.2±32.3    | 61.5±27.4   |
| Spleen         | 47.8±21.98                        | 63.4±23.1    | 73.7±55.4   |

Table 2. Histamine release from mast cells by incubation with compound 48/80 or polymyxin B for 5 min at 37°C

|                | Histamine (µg/10⁶ MC) | Treatment        | Histamine release (%) |
|----------------|-----------------------|------------------|-----------------------|
| Control        | 11.77±0.93            | Compound 48/80   | 2.36±1.42             |
|                |                       | Polymyxin B      | 92.15±4.83            |
|                |                       |                  | 83.40±15.10           |
| Mg-deficient*  | 19.36±4.95            | Compound 48/80   | 2.24±2.66             |
|                |                       | Polymyxin B      | 54.05±7.94            |
|                |                       |                  | 25.60±12.47           |

Compound 48/80 (0.5 µg/ml) or polymyxin B (5 µg/ml) was used. Each value represents the mean of six to ten experiments. *On the 8th day of Mg depletion.
of the spleen, skin and peritoneal mast cells were compared between the control and Mg-deficient rats (Fig. 2). The HDC activity in the spleen increased gradually from the 4th day until the 8th day of Mg depletion.

In the skin of Mg-deficient rats, the HDC activity was quite high on the 4th day; but on the 6th day, it became lower than the level on 4th day and again increased gradually. These results are similar to those reported previously (1). On the 8th day of Mg depletion, the HDC activity of peritoneal mast cells from the Mg-deficient rats was 2 times as high as that of the activity in the control rats.

The relationship between the HDC activity and the number of spleen cells is shown in Fig. 3. The HDC activity of the spleen cells isolated from Mg-deficient rats was about 30 times as high as that of the controls.

### Discussion

A significant increase in the histamine content was observed in the spleen on the 8th day of Mg deficiency. However the histamine content of the skin containing numerous mast cells showed no significant change until the 8th day of Mg deficiency. These results are consistent with our previous observation (2). The histamine content in the peritoneal mast cells on the 8th day of Mg deficiency was 2 times higher than that of the control rats. It appears that the effects

### Table 3. Histamine release from spleen cells by incubation with compound 48/80 or polymyxin B for 5 min at 37°C

| Treatment          | Histamine release (%) |
|--------------------|-----------------------|
| Control            | 5.28 ± 3.84           |
| Compound 48/80     | 11.45 ± 4.35          |
| Polymyxin B        | 14.42 ± 14.86         |
| Mg-deficient*      | 1.94 ± 2.78           |
| Compound 48/80     | 13.89 ± 8.34          |
| Polymyxin B        | 7.72 ± 1.20           |

Compound 48/80 (0.5 μg/ml) or polymyxin B (5 μg/ml) was used. Each value represents the mean of six to ten experiments. *On the 8th day of Mg depletion.
of Mg deficiency on cutaneous mast cells are different from those on the peritoneal mast cell.

We previously reported that on the 8th day of Mg depletion, few mast cells were found in the spleen of the control and Mg-deficient rats. Elliott (13) also reported that no mast cells were present in the spleen of rats. Hence it is assumed that there may be no direct relationship between the increase in the histamine content of the spleen caused by the Mg depletion and the number of mast cells in this organ.

Systemic administration of compound 48/80 or polymyxin B caused the release of histamine from the skin of the control as well as Mg-deficient rats, and the extent of the histamine release in the Mg-deficient rats was less than that of the control rats. The histamine content in the spleen of the control rats was increased by the administration of both the histamine releasers. This phenomenon suggests the importance of histamine uptake or other unknown mechanisms in the regulation of histamine level in this organ. In the Mg-deficient rats, however, the histamine content of the spleen was decreased by these releasers; hence the supposition is that some parts of the splenic histamine are sensitive to the releasers. The effects of these releasers on the splenic histamine seem to be quite complex, but at least, it is certain that the splenic histamine storage in the Mg-deficient rats may be different from that of the control rats.

In in vitro experiments, compound 48/80 or polymyxin B caused histamine release from peritoneal mast cells isolated from the control as well as Mg-deficient rats. The extent of histamine release from these cells was less in the Mg-deficient rats than in the control rats. These data are similar to those obtained in in vivo experiments in the skin. Since the skin and the peritoneal mast cells obtained from the Mg-deficient rats are less sensitive to these releasers, it may be reasonably assumed that these mast cells are deficient in or devoid of the appropriate receptors for these histamine releasers or that some parts of the histamine pool are insensitive to these drugs.

The spleen cells isolated from the control and the Mg-deficient rats were less responsive to compound 48/80 or polymyxin B than the mast cells (Table 3). Such a low response to histamine releasers is similar to that of immature mast cells (14). Other histamine containing cells such as enterochromaffin-like cells (15), parietal cells (16) and basophilic leukemia cells (17) are also insensitive to these releasers. As suggested by Erjavec (18) and Pearce (19), concerning the responsiveness to histamine releasers, there may be a difference between spleen cells and peritoneal mast cells. Thus, viewed from the release caused by these histamine releasers, the histamine storage in spleen cells is obviously different from that in peritoneal mast cells.

The HDC activity in the spleen cells isolated from the Mg-deficient rats increased markedly on the 8th day of Mg depletion, but the activity in peritoneal mast cells increased only slightly. Hence, the high histamine content of the spleen cells may be due to their high HDC activity. However, what types of cells in the spleen of Mg-deficient rats have the high HDC activity and histamine content remains to be clarified.

The control rat showed low HDC activity in the spleen with a great deal of variation from animal to animal. Yamada et al. (20)
reported that in the assay of HDC activity in a crude extract of the spleen, the presence of protease inhibitors was essential. No protease inhibitors were used in this experiment, so one of the reasons for the variation in HDC activity of the spleen may due to the variation in protease activity. In the case of peritoneal mast cells, Beaven et al. (21) reported that histamine production by intact peritoneal mast cells was 10 to 30 times greater than that of disrupted cells. So the HDC activity may be extremely labile after cellular integrity is lost. It seems necessary to examine these possibilities.

From these results, there seems to be no direct relationship between the splenic histamine content and mast cell density.

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