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INDUCTION OF HISTIDINE DECARBOXYLASE ACTIVITY IN THE SPLEEN OF MICE TREATED WITH STAPHYLOCOCCAL ENTEROTOXIN A AND DEMONSTRATION OF ITS NON-MAST CELL ORIGIN

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Histidine decarboxylase (HDC) activity increased 13-, 7-, and 2-fold in the spleen, lung and liver, respectively, but not in other tissues of C57BL/6 mice injected i.v. with 50 μg/kg of Staphylococcal enterotoxin A (SEA). But even in the spleen, increase in the histamine level was only 1.5 times that of untreated mice. In genetically mast cell-deficient $WBB6F_1$- W/W^V mice HDC activity in the spleen increased to the same extent as in wild type $WBB6F_1$- +/+ mice on SEA treatment, but the histamine level in the spleen also increased 20-fold, whereas it increased only 1.4-fold in +/+ mice. These results suggest that the increases in HDC and histamine resulted from interaction of SEA with non-mast cells in tissues.

Staphylococcal enterotoxin A (SEA) is known to have strong mitogenic activity on T cells as well as being an enteric toxin (1,2). It also has immunomodulating activity in *in vitro* and *in vivo* immune systems (3-6), but the mechanism of its action is unclear, although some mediators, such as interferon, have been suggested to be involved in its effects. There are many reports that histamine, a potent biogenic amine, is a mediator in the

Abbreviations: HDC, histidine decarboxylase, L-histidine carboxylyase, E.C.4.1.1.22; SEA, Staphylococcal enterotoxin A.

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immune system (7-9), and that the administrations of some mitogenic substances, such as Escherichia coli lipopolysaccharide and concanavalin A, induce HDC and increase the histamine level in some tissues (10-12). Thus, histamine may be involved in the immunomodulating activity of SEA.

In this paper, we report that SEA caused marked induction of HDC in the spleen and lung of mice. We also show that this increase of HDC activity was observed in genetically mast cell-deficient $WBB6F_1$- W/W^V mice (13), and that increase in the histamine level was clearly demonstrated in these W/W^V mice, whereas the increase was obscured by the initial high level of histamine in other mice.

MATERIALS AND METHODS

Treatment of Mice with SEA: Female C57BL/6 mice (7-8 w) were injected i.v. with 50 μg/kg of SEA, purified in our laboratory by the method of Shinagawa et al. (14). The mice were killed by decapitation at the indicated times after the injection. Various tissues were excised as quickly as possible, and stored at -80°C until use. Control mice received the same amount of the vehicle (ca. 100μl saline). Female $WBB6F_1$- +/+ and -W/W^V mice, raised in our laboratory, were also treated with SEA or vehicle as described above.

Biochemical Measurements: For HDC assay, tissues (50-200 mg) were homogenized with 0.5 ml of cold Solution A [0.1 M potassium phosphate buffer(pH 6.8), 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate, 1 % polyethylene glycol (average molecular weight 300, w/w), and 0.5 μg/ml each of leupeptin and chymostatin] in a Polytron homogenizer (Kinematica, Lucern, Switzerland) operated at the maximum setting for two 10-sec periods. The homogenate was centrifuged at 10,000 x g for 20 min and the supernatant was dialyzed three times against 100 volumes of Solution A. HDC activity was assayed as described previously (15). In brief, the dialyzate was incubated with 0.25 mM histidine for 15 h and histamine was separated from histidine on a short column of Amberlite CG-50. Histamine was measured fluorometrically by the o-phthalaldehyde method (16) as described by Watanabe et al. (15). The addition of protease inhibitors or aminoguanidine (2.5 mM) to the reaction mixture did not significantly affect the results and so in most experiments these reagents were omitted. For histamine assay, about half the spleen was homogenized with 1.0 ml of 3 % perchloric acid. The homogenate was centrifuged as described above and the supernatant was obtained. Histamine was measured fluorometrically by the o-phthalaldehyde method (16) in an HPLC system as described by Yamatodani et al. (17). Protein was measured by the method of Bradford (18) with bovine IgG as a standard (Bio-Rad Lab., Richmond, Calif., USA).

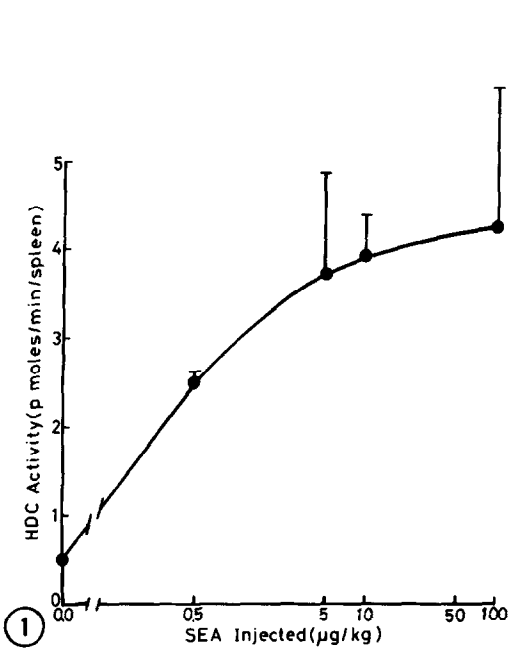
RESULTS

HDC Activity in Various Tissues of Mice Injected with SEA : Table I shows the HDC activities of various tissues of mice injected with SEA or saline. Five hours after the injection, HDC activities in the spleen, lung and liver had increased 13-, 7- and 2-fold, respectively, but except for a slight increase in the thymus HDC activity did not increase in other tissues. HDC activity in the spleen reached a peak 5 hr after SEA injection and decreased gradually from 8 hr after the injection (data not shown). The histamine content of the spleen increased only 1.5-fold (280 and 408 pmoles in control and treated spleens, respectively). Figure 1 shows the dose-response curve to SEA of induction of HDC activity. Since the induction was maximal at a

Table I: Effect of Intravenous Injection of SEA on HDC Activity in Various Tissues of C57BL/6 Mice

Tissue	HDC Activity (pmoles/min/organ or mg protein)		
	Untreated	SEA treated	Fold Increase
Brain	1.74 ± 0.98	1.80 ± 0.43	1.03
Lung	0.34 ± 0.15	1.96 ± 0.76	6.80**
Liver*	0.057 ± 0.040	0.107 ± 0.033	1.70**
Kidney	2.80 ± 1.04	4.19 ± 1.89	1.50
Blood*	0.17 ± 0.06	0.20 ± 0.28	1.18
Thymus	0.51 ± 0.43	1.61 ± 1.48	3.15
Mesentric lymph node	0.36 ± 0.32	0.28 ± 0.19	0.78
Spleen	0.61 ± 0.29	8.03 ± 1.34	13.1***
Stomach	4.91 ± 1.58	3.76 ± 4.15	0.77

One μg of SEA was injected i.v. into C57BL/6 mice and 5 hr later the mice were killed. HDC activity in the supernatants of homogenates of various tissues was assayed as described in MATERIALS AND METHODS. Values of HDC are means ± S.D. from 4 mice. * per mg protein, ** $p < 0.05$ by Student's t-test. *** $p < 0.001$ by Student's t-test.



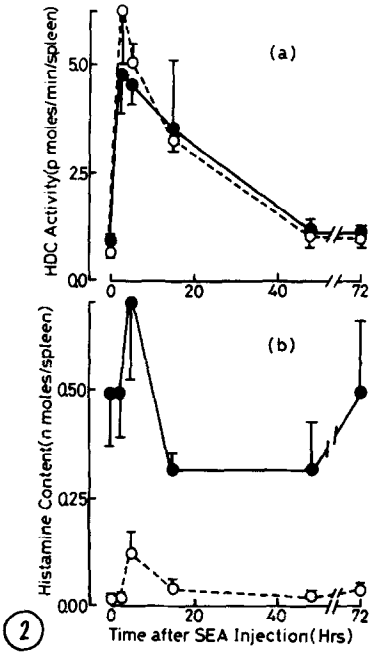


Fig. 1. Dose-Response Curve of HDC Induction in Mouse Spleen by SEA. C57BL/6 mice were injected i.v. with various amounts of SEA and killed 5 hrs later. HDC activity in the supernatants of homogenates of spleen was assayed as described in MATERIALS AND METHODS. Bars show S.D. (n=5).

Fig. 2. Time Course of HDC Induction in Mouse Spleen by SEA. Female WBB6F$_1$-+/+ and -W/W^V mice were injected with 1.0 μg of SEA and killed at the indicated times. HDC activity and histamine content of their spleen were measured as described in MATERIALS AND METHODS. Bars show S.D. (n=4-5). ——— , +/+ mice ; ------, W/W^V mice.

dose of 50 μg/kg of SEA, this dose was used throughout this work.

Induction of HDC and Increase of Histamine in WBB6F$_1$- +/+ and W/W^V Mice by SEA: To examine whether HDC induction by SEA involved mast cells or non-mast cells, we used genetically mast cell-deficient WBB6F$_1$- W/W^V mice (13). Figure 2 shows the time courses of increase in HDC activity and the histamine level in the spleens of WBB6F$_1$- +/+ and -W/W^V mice after SEA injection. In the spleen of WBB6F$_1$- W/W^V mice, HDC activity increased to almost the same extent as in control +/+ mice 5 hr after SEA injection. The histamine content of the spleen of W/W^V mice also increased in parallel with increase in HDC activity. The amount

of increase in histamine content in the spleen of +/+ mice was almost the same as that in $\underline{W/W^V}$ mice, but due to the initial high level of histamine in mast cells the increase was only 1.4-fold that of controls. These results indicate that histamine induced by SEA was derived from non-mast cells.

DISCUSSION

The present study demonstrated that SEA had high activity for induction of HDC in the spleen when administered i.v. to mice. In the spleen, histamine increased to a maximum 5 hrs after the injection, and inductions of HDC and histamine were both apparent in mast cell-deficient $WBB6F_1$-$\underline{W/W^V}$ mice*. These results suggest that HDC-induction by SEA was due to cells other than mast cells. The presence of non-mast cells containing histamine has been known for a long time, and was clearly shown in various tissues of $WBB6F_1$-$\underline{W/W^V}$ mice by Yamatodani et al.(17). The induction of HDC in non-mast cells in the skin of the $\underline{W/W^V}$ mice was also demonstrated by application of a tumor promoter, 13-O-tetradecanoyl phorbol-12-acetate (19). The demonstration of induction of HDC by SEA in the present study provides further evidence for induction of HDC in non-mast cells.

Previously we showed that SEA has an immunosuppressive effect on antibody formation and delayed hypersensitivity in mice (6). We also showed that spleen cells from SEA-treated mice have a suppressor function (6). Since there are many reports of histamine-activated suppressor cells (20,21), we suggest that these immunosuppressive effect of SEA involve histamine as a mediator. Recently, Endo reported that mitogenic substances such as lipopolysaccharide and concanavalin A induced HDC in some

* Increase of HDC activity by E. coli endotoxin was also observed in $\underline{W/W^V}$ mice (Dr. K. Nakano, Nagoya University ; personal communication).

lymphatic tissues of mice 4.5 hrs after their injection (11), and since he also observed these inductions in nude mice, he suggested that T cells were not necessary for HDC induction (12). The non-mast cells having HDC in the spleen of $WBB6F_1$-W/W^V as well as in normal mice require identification.

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