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EFFECT OF α-FLUOROMETHYLHISTIDINE, A SUICIDE INHIBITOR OF HISTIDINE DECARBOXYLASE, ON HISTAMINE LEVELS IN MOUSE TISSUES

KAZUTAKA MAEYAMA,* TAKEHIKO WATANABE, YOSHITAKA TAGUCHI, ATSUSHI YAMATODANI and HIROSHI WADA

Department of Pharmacology II, Osaka University School of Medicine, Kita-ku, Osaka 530, Japan

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Abstract—The effects of α-fluoromethylhistidine (α-FMH), a new suicide inhibitor [Kollonitsch *et al.*, *Nature, Lond.* **274**, 906 (1978)], on histidine decarboxylase (HDC) activities and histamine contents of the skin, fundic stomach and brain of mice were investigated. Four hours after i.p. administration of α-FMH to ddy mice, HDC activities in the brain, stomach and skin had decreased in a dose-dependent way (1–25 mg/kg), by a maximum of 90–95%. The histamine levels in the brain and stomach decreased to 50% of the control levels, whereas the level in the skin did not change at all. The time courses of changes in HDC activities and histamine levels were examined. After i.p. administration of 25 mg/kg of α-FMH, HDC activities in these tissues dropped rapidly within 1 hr. Recovery of HDC activities in the stomach and skin began within 12 hr, but the activity in the brain remained low for 24 hr, confirming the result of Garbarg *et al.* [*J. Neurochem.* **35**, 1045 (1980)]. The histamine content of the stomach decreased to 40% of the original level in 8 hr and recovered within 12 hr, whereas that in the brain decreased to 50% and remained low for more than 24 hr. The histamine content of the skin did not change. These results suggest that the histamine level that was not reduced by α-FMH was derived from mast cells. During the above experiments, no behavioral changes of the animals were detected. α-FMH prevented the increase in HDC activity in mouse kidney on day 18 of gestation when administered i.p. every 12 hr from day 13. No abnormalities were seen in fetuses and neonates after this treatment. It is concluded that α-FMH causes depletion of newly synthesized histamine *in situ* and, thus, is useful for studies on histamine.

Histidine decarboxylase (HDC, L-histidine carboxylyase, EC 4.1.1.22) is the only enzyme involved in the formation of histamine from the precursor amino acid, histidine. Therefore, histamine production *in vivo* can be regulated either by the activity of HDC or by the concentration of L-histidine. Since histamine is concerned with various physiological and pathological conditions, such as allergic reactions, inflammation, gastric acid secretion, neurotransmission and certain kinds of rapid tissue proliferation [1–5], a number of HDC inhibitors have been developed and used *in vivo* to lower the tissue histamine content [6–11]. However, most of them are not as effective *in vivo* as expected from *in vitro* studies, mainly due to their low potencies and specificities. Thus, new HDC inhibitors with strong potency and high specificity are required.

Recently, Kollonitsch *et al.* [12] synthesized a series of α-fluoromethyl amino acids and showed that they are very strong specific inactivators of the corresponding amino acid decarboxylases. From kinetic analyses of their modes of action, these compounds are considered to be suicide inactivators of decarboxylases [13, 14].

One of them, [*S*]-α-fluoromethylhistidine (α-FMH), is a promising candidate for *in vivo* studies on histamine formation. Schwartz and co-workers recently found that, in mouse brain, the administration of α-FMH caused a marked decrease in HDC activity and a concomitant decrease in the histamine level, though the decrease was not as great as that of HDC [15]. They suggested that the histamine that was not depleted by α-FMH was derived from mast cells.

In this work, we used α-FMH to study the HDC and histamine levels in peripheral tissues and brain of mice and found that this compound was useful for depletion of histamine from non-mast cells.

MATERIALS AND METHODS

Administration of α-FMH to mice. Male ddy mice (8 weeks old), weighing 22 ± 2 g, were purchased from the Kansai Experimental Animal Research Institute, Osaka, Japan, and kept in groups in a room at 25° with light and dark cycles of 12 hr. For 12 hr before sacrifice no food was given, but water was supplied *ad lib.* A solution of α-FMH in 0.9% NaCl was injected i.p. into mice, and the same volume of the vehicle (0.1 ml) was given to control animals. Each group consisted of five mice. Experiments were started at 10:00 a.m. and, at appropriate times after a constant dose of α-FMH, mice were killed by cervical dislocation, and their brains, *ca.* 2 cm^2 of their skin, and their fundic stomachs were removed as quickly as possible. The tissues were weighed and used immediately or stored at −80°

* Address all correspondence to: Dr. Kazutaka Maeyama, Department of Pharmacology II, Osaka University School of Medicine, 3-57 Nakanoshima 4-chome, Kita-ku, Osaka 530, Japan.

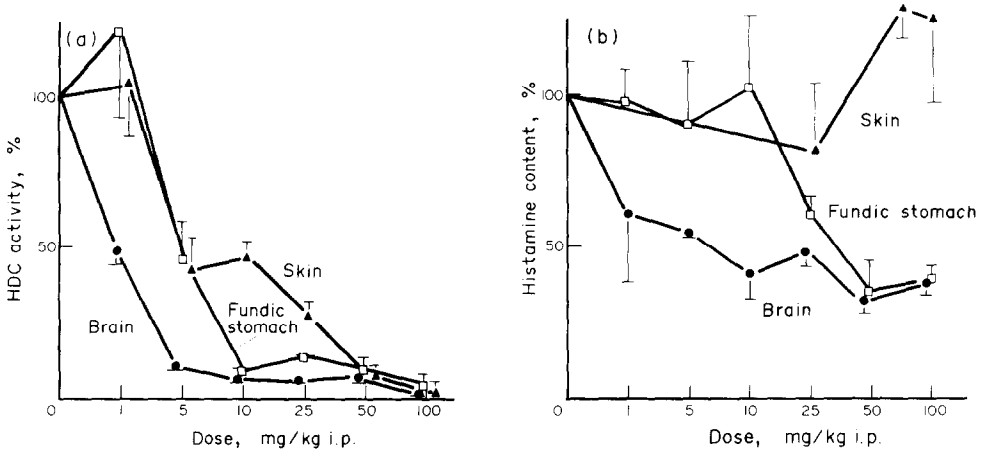

Fig. 1. Effect of dose of α-FMH on HDC activity and histamine content of skin, somach and brain of mice. Mice were given α-FMH (1–100 mg/kg) i.p. and killed 4 hr later. HDC activities and histamine contents of skin, stomach and brain were analyzed as described in Materials and Methods. (a) The HDC activities of skin, stomach and brain of control mice, taken as 100%, were 0.26 ± 0.04, 4.01 ± 0.65 and 0.22 ± 0.02 pmoles per min per mg protein respectively (N = 7). (b) The histamine contents of skin, stomach and brain from control mice, taken as 100%, were 138 ± 10, 117 ± 19 and 0.40 ± 0.04 nmoles per g wet weight respectively (mean ± S.E.).

until use. α-FMH (25 mg/kg) was also given i.p. to pregnant mice twice a day from day 13 of pregnancy to parturition. On day 18, mice were killed 8 hr after the last injection of α-FMH, and their kidneys were stored at −80° until use.

Histidine decarboxylase assay. HDC was extracted from mouse tissues and assayed as described previously [16, 17]. Tissues were homogenized in 2.0 ml of Solution A using a Polytron (Kinematica, Switzerland) operated at the maximum setting for two 10-sec periods in an ice bath. Solution A consisted of 0.1 M potassium phosphate buffer, pH 6.8, 0.01 mM pyridoxal-5'-phosphate, 0.2 mM dithiothreitol, 1.0% polyethylene glycol (average relative molecular weight, 300) and 2 μg/ml each of pepstatin, chymostatin, leupeptin, and antipain (Protein Research Foundation, Minoh, Osaka, Japan). The homogenate was centrifuged at 10,000 *g* for 20 min, and the resulting supernatant fraction was dialyzed twice against 100 vol. of Solution A at 4°. HDC was assayed as described previously in the presence of 0.25 mM L-histidine [16, 17].

Protein determination. The protein content of the supernatant fraction obtained as described above was measured by the method of Lowry *et al.* [18] with bovine serum albumin as a standard.

Histamine analysis of tissue extract. Tissues were homogenized in 2.0 ml of 5% perchloric acid–5 mM EDTA with a Polytron, as described above, and centrifuged. The resulting supernatant fraction was obtained as above and analyzed for histamine as described previously [19]. Histamine was purified by Bio-Rad AG-50 (Bio-Rad, Richmond, VA, U.S.A.) column chromatography and high-performance liquid chromatography (TSK, IEX 510 SP, Toyo Soda Ltd. Co., Tokuyama, Japan), and measured by the *o*-phthalaldehyde method of Shore *et al.* [20], using the autoanalyzer system developed in this laboratory [19].

Measurement of α-FMH in kidney. For determination of α-FMH concentrations, kidneys were homogenized in 2 ml of 3% trichloroacetic acid as above, and the supernatant fraction was extracted with 2 ml of ethyl acetate. The pH of the water phase was increased to *ca.* 12 by addition of 2 M LiOH, and the solution was lyophilized. The residue was dissolved in 0.2 M sodium lithium buffer, pH 2.2, and promptly analyzed in a Hitachi amino acid analyzer model 835. α-FMH was eluted a little faster than ammonia, and its color value was 62% of that of histidine. The recovery of α-FHM in these procedures was 83%.

RESULTS

Effect of the dose of α-FMH on the HDC activity and histamine content of skin, stomach and brain. Panels a and b of Fig. 1 show the effects of dose of α-FMH on the HDC activity and histamine content, respectively, in skin, fundic stomach, and brain of mice 4 hr after treatment. As shown in Fig. 1a, the HDC activities in these tissues decreased dose dependently and reached minima (10% of the original activities) with 50, 10 and 5 mg/kg of α-FMH respectively. HDC in the skin was rather resistant to α-FMH; at a dose of higher than 50 mg/kg, HDC activities of the brain, stomach and skin were all suppressed.

The changes in histamine levels in these tissues were different from those in HDC activities (Fig. 1b). The histamine levels in brain and stomach were decreased dose dependently by α-FMH but reached only 40% of the control levels, even with doses which decreased the HDC activities to 10% of the control levels; the histamine level of the skin was not decreased. Increase in the dose of α-FMH to 100 mg/kg did not cause a further decrease in the histamine levels.

Time-dependent changes in the HDC activity and histamine level of the brain, stomach and skin of mice treated with α-FMH. Figure 2 shows the time courses of changes in HDC activities and histamine levels after i.p. administration of 25 mg/kg of α-FMH to

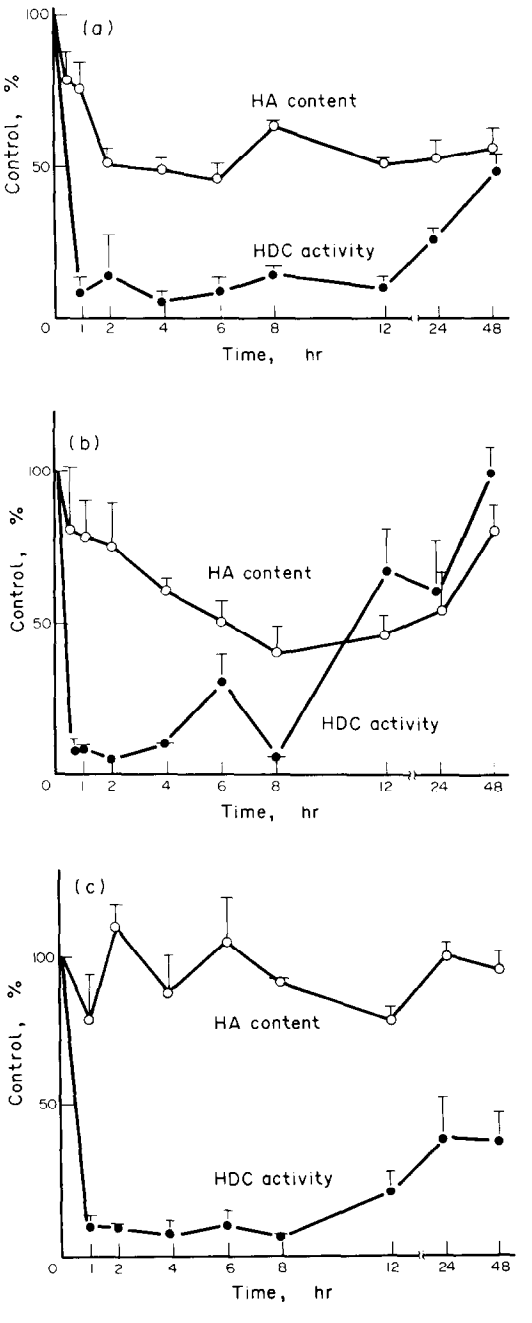

Fig. 2. Time-dependent changes in HDC activities and histamine levels of brain, stomach and skin of mice treated with α-FMH. Mice were given α-FMH (25 mg/kg) and killed at the indicated times. HDC activity and histamine content were measured as described in Materials and Methods. The HDC activities of brain (a), stomach (b), and skin (c) of control mice, taken as 100%, were 0.20 ± 0.01, 3.59 ± 0.43 and 0.33 ± 0.07 pmoles per min per mg protein; their histamine levels, taken as 100%, were 0.39 ± 0.04, 122 ± 9 and 144 ± 9 nmoles per g wet weight respectively (mean ± S.E.).

mice. In the three tissues, the HDC activity decreased very rapidly, reaching a minimum in 1 hr. The activity in the brain (Fig. 2a) remained low until 12 hr and then increased slowly to the original level after 96 hr, confirming the results of Garbarg *et al.* [15]. In the stomach (Fig. 2b), a slight and inconstant increase in the activity was observed at about 6 hr, but then the activity became low again; it began to increase after 12 hr more rapidly than that of the brain, reaching nearly the control level after 48 hr. In contrast to HDC activity, the histamine level in the brain decreased in 2 hr to, and remained at, 50% of the initial levels for 48 hr (Fig. 2a). In the stomach, the histamine level decreased gradually over 8 hr and began to increase at 12 hr. No increase in histamine content was observed in the stomach at about 6 hr when the HDC activity had partially recovered (Fig. 2b). In the skin, HDC activity decreased to 10% of the control level in 1 hr, and remained low for 48 hr (Fig. 2c), but the histamine level did not decrease significantly during this period (Fig. 2c). When α-FMH at a dose of 100 mg/kg was administered i.p. to mice, kidney tissue levels of α-FMH (data not shown) increased to maxima in 1 hr (i.e. 220 nmoles/g of kidney) and then decreased rapidly to lower levels in 4 hr (i.e. less than 20 nmoles/g of kidney).

Effect of α-FMH on HDC activity in the kidney of pregnant mice. As one application of α-FMH to studies *in vivo* on physiological and pathological roles of histamine, we examined the effect of α-FMH on the increase in the kidney HDC activity observed in pregnant mice [2, 3]. α-FMH was given to pregnant mice twice a day, this dose schedule being chosen on the basis of the rapid elimination of the compound described above. Table 1 shows that α-FMH completely prevented the increase in kidney HDC activity of mice on day 18 of pregnancy. The HDC activity of kidney from non-pregnant female mice was 2.4 ± 1.5 pmoles per min per mg protein. The histamine content was also markedly reduced by this treatment. No abnormality in the number of embryos or their viability was observed after this treatment.

DISCUSSION

These studies have shown that α-FMH, a new suicide inactivator of histidine decarboxylase [12], was effective *in vivo* not only in brain, as reported by Garbarg *et al.* [15], but also in peripheral tissues. When administered i.p. to mice, it caused rapid, marked and prolonged inactivation of HDC in the tissues examined and also decreased the histamine level to half the control level in the brain and stomach, but it did not affect the level in the skin (Figs. 1 and 2). Since no further decrease in histamine level was observed at higher doses of the compound, these partial decreases in the brain and stomach and the absence of a decrease in the skin must reflect the nature of histamine storage. Evidence is accumulating that histamine is present not only in mast cells but also in non-mast cells [1–5]. Mast-cell histamine is stored in granules and is thus protected from release and metabolic degradation [21], whereas non-mast-cell histamine is not stored but is released by stimuli and metabolized further [1–3]. We found that the brain and stomach have definite amounts of non-mast-cell histamine, using W/W^v mice that have been shown by Kitamura *et al.* [22]

Table 1. Effect of α-FMH on HDC activity and histamine content of kidney tissue of mice on day 18 of pregnancy*

	N	HDC activity (pmoles/min/mg protein)	Histamine content (nmoles/mg protein)
Control	4	43.0 ± 10.2	0.40 ± 0.09
Treated†	4	1.51 ± 0.56	0.021 ± 0.008

* Values are averages ± S.D. N is the number of mice.
† Mice were given 25 mg/kg α-FMH, i.p., twice a day from day 13 of pregnancy, and were killed on day 18. The kidneys were combined, and their HDC activity and histamine content were measured as described in Materials and Methods.

to be devoid of mast cells [17, 23]. The present results showing partial decrease in the histamine level in the brain and stomach and no change in the level in skin are consistent with the distribution of mast cells in these tissues. Therefore, it is concluded that α-FMH inactivates histidine decarboxylases in both mast cells and non-mast cells, but that histamine in mast cells does not decrease rapidly on i.p. administration of the compound. It may be possible to deplete mast cells of histamine by prolonged administration for several weeks.

Thus, α-FMH is a useful compound for depleting non-mast cells of histamine and provides another experimental tool for studying histamine in physiological and pathological conditions. As one example of its application in *in vivo* studies, we found that α-FMH completely prevented the increase in HDC activity normally observed in kidney from pregnant mice [2]. With respect to the functions of histamine, it will be particularly interesting to test the effect of α-FMH on gastric acid secretion and on behaviors such as intake of food and water, thermoregulation, and the sleep-waking cycle of animals.

Although a single administration of α-FMH does not affect the histamine level of mature mast cells, it will also be interesting to test its effect on histamine levels under certain conditions where mast cells begin to proliferate, or increase in number, such as during growth of rodent embryos and so on. We have shown that tetradecanoylphorbolacetate increases the HDC activity when applied to mouse skin [23]. The effect of pretreatment of mice with α-FMH on this HDC increase is now under investigation.

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