Effects of α-Fluoromethylhistidine on Increase in Histidine Decarboxylase Activity of Maternal Mouse Kidney Observed during Late Pregnancy and Evidence for Its Non-mast Cell Origin by Using Estrogen and W/Wv Mice

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Abstract—The increase of histidine decarboxylase (HDC) activity during late pregnancy in the whole bodies of fetal mice and the kidneys of their mothers were almost completely inhibited by i.p. administration of 25 mg/kg of α-fluoromethylhistidine (α-FMH), a suicide inhibitor of HDC, starting on day 13 of pregnancy. The increase of HDC in fetal mice was previously shown to be in mast cells [T. Watanabe et al., Proc. Natl. Acad. Sci. U.S.A. 78, 4209-4212 (1981)]. The increase of HDC in maternal kidneys was examined by using estrogen and W/Wv mice, which were devoid of mast cells and infertile. Treatment of castrated mice with 17β-estradiol increased the HDC activity of the kidney, and this increase was antagonized by concomitant treatment with clomiphene, an antiestrogen, confirming that the increase is mediated through an estrogen receptor. HDC activity in the kidney of W/Wv mice was also increased by estradiol treatment, indicating that HDC activity was associated with non-mast cells.

The activity of histidine decarboxylase (HDC, L-histidine carboxylase, E.C. 4.1.1.22) increases greatly under various conditions such as on treatment with hormones (e.g., gastrin, estrogen and thyroxine) or chemical agents (e.g., 13-O-tetradecanoyl phorbol-12-acetate and endotoxin) and during rapid growth (e.g., during pregnancy and wound healing and in tumors) and hypersensitive reactions, which are reviewed in Ref. (1-6). Accordingly, as HDC is a rate limiting enzyme in histamine formation, the histamine content also increases in situ under these conditions and histamine acts as an autacoid. However, the precise function of histamine in most conditions mentioned above is still uncertain (1-4).

One approach in studies on the action of histamine in physiological and pathological conditions is to use a specific inhibitor of HDC, which stops de novo synthesis of histamine and lowers its level: the resulting changes caused by histamine depletion should then suggest the role of histamine in the particular case. α-Fluoromethylhistidine (α-FMH) is a potent and specific inhibitor of HDC in vitro (7), and the mechanism of its action is well known (8, 9). It is a type of inhibitor called a $K_{cat}$ inhibitor (10), suicide...
substrate (11), or enzyme-activated irreversible inhibitor (12). Moreover, \(\alpha\)-FMH is a strong and specific inhibitor in vivo, as expected from in vitro studies (8, 13, 14). Thus, it was interesting to examine the effect of \(\alpha\)-FMH administration on the increase of HDC activity and the resulting depletion of histamine on the conditions described above.

It is known that HDC activity increases greatly in whole fetuses (15) and maternal kidney (1) several days before parturition. We showed that the increase of HDC activity and histamine in whole bodies of mouse fetuses during later pregnancy derived from mast cells, because \(W/W\) mice, which were shown by Kitamura et al. (16) to be devoid of mast cells, showed no increase in HDC activity and histamine (17). In the present work, we studied the effect of administration of \(\alpha\)-FMH on the increase of HDC activity during pregnancy, and we found that HDC activity was not increased by \(\alpha\)-FMH treatment in either fetuses or the kidney of pregnant mice and that administration of \(\alpha\)-FMH during late pregnancy did not affect the maternal or fetal side, confirming the results reported by Barthoileyns and Bouclier (18). We examined whether the increase of HDC activity in maternal kidneys derived from mast cells or non-mast cells by using a model system, i.e., the induction of HDC in the kidney of \(W/W^v\) mice treated with estrogen, because \(W/W^v\) mice were infertile. We also found that the increase in HDC activity in mouse kidney was mediated by an estrogen receptor and we showed by using \(W/W^v\) mice that this increase occurred in non-mast cells.

Materials and Methods

Treatment of mice with drugs: Pregnant and male ddY mice were purchased from the Kansai Experimental Animal Research Institute, Osaka, Japan. Pregnant mice were given 25 mg/kg of \(\alpha\)-FMH, i.p., twice a day from day 13 of pregnancy. On appropriate days, they were killed by decapitation, and the kidneys and the fetuses were excised. Male mice (20 g body weight) were castrated under ether anaesthesia and 7 and 8 days later and 20 and 21 days later were given s.c. 0.2 mg 17-\(\beta\)-estradiol and 0.5 mg testosterone, respectively. Some mice were treated with 0.2 mg of clomiphene, i.p., every day throughout the experiments. On the indicated days, they were killed by decapitation, and their kidneys were excised. \(WBB6F_1-W/W^v\) mice (male, 20 g body weight, Jackson Lab., Bar Harbor, Maine, U.S.A.) were given 0.2 mg of 17-\(\beta\)-estradiol and 0.5 mg of testosterone, s.c., on days 0 and 1 and days 33 and 34, respectively, and were killed by decapitation on day 35 and their kidneys were excised. Materials were stored at \(-80^\circ\)C until use.

Extraction of enzymes and histamine: Mouse whole fetuses or maternal kidneys were homogenized in 5 volumes of Solution A [0.1 M potassium phosphate buffer, pH 6.8, 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate and 1% (w/w) polyethylene glycol (average molecular weight, 300)] in a Polytron homogenizer (Kinematica, Luzern, Switzerland) operated at the maximum setting for two 10-sec periods in an ice-bath (19). Nine-tenths of the homogenate was centrifuged at 10,000 \(\times\) g for 20 min, and the supernatant was dialyzed overnight against 2 changes of 100 volumes of Solution A.

Assays of HDC and DOPA decarboxylase activities: HDC and DOPA decarboxylase activities were assayed as described before (17, 20). Briefly, extracts were incubated with 0.25 mM L-histidine or L-DOPA in Solution A for 60 min at 37°C, and histamine or dopamine was separated from the substrates by Amberlite CG-50 column chromatography. Histamine and dopamine were measured fluorometrically by the o-phthalaldehyde method (21) in an autoanalyzer developed in this laboratory (20) and by the ethylene diamine condensation method (22), respectively.

Protein measurement: Protein was measured by the method described by Lowry et al. (23) with bovine serum albumin as the standard.

Histamine analysis: One-tenth of the homogenate was mixed with 9 volumes of 0.6 M perchloric acid and was used for histamine determination as described...
previously (17, 24). Briefly, the above mixture was centrifuged, and the supernatant was applied to an Amberlite CG-50 column. The histamine fraction was subjected to high performance liquid chromatography, and histamine was measured fluorometrically by the o-phthalaldehyde method (21) as described previously (24).

Reagents: α-FMH was a gift from Dr. J. Kollonitsch, Merck Sharp and Dohme Research Laboratories, Rahway, N.J., U.S.A. Other chemicals were obtained from commercial sources.

Results

Effect of α-FMH on increase of HDC activities and histamine levels of whole mouse fetuses and maternal kidneys during late pregnancy: The HDC activity of mouse whole fetuses increased greatly towards the end of gestation, reaching a peak on day 18 and decreasing sharply after parturition (Fig. 1) as described previously (1, 2, 17). The histamine level increased and decreased in parallel with the HDC activity, although it was more retarded than HDC (Fig. 1) (17). When pregnant mice were given 25 mg/kg of α-FMH twice a day starting on day 13 of pregnancy, the increase of HDC activity was completely blocked, and the histamine level also remained low, although its increase was not blocked completely like that of HDC.

The HDC activity of maternal mouse kidney increased during late pregnancy, reaching a peak on about day 18 of pregnancy and then decreasing (Fig. 2), as described by Kahlson and Rosengren (1, 2). Administration of α-FMH completely blocked the increase of HDC activity. On day 18 of pregnancy, the histamine content of the kidney of untreated pregnant mice was 0.40 nmole/mg protein, while that of α-FMH-treated mice was 0.021 nmole/mg protein.

However, the number and size of newborns from α-FMH-treated mice were almost the same as those from untreated ones, and no abortions or abnormalities were observed. The behaviors of mother mice treated with α-FMH seemed to be normal on appearance.

Increase in HDC activity of kidneys of mice treated with estradiol: W/WV mice, which were shown by Kitamura et al. (16) to be devoid of mast cells, should be useful for determining whether the increase in HDC activity described above was due to mast cells or non-mast cells. Our previous studies showed that the increase in HDC activity and histamine levels of the fetal side derived from mast cells (17). Thus, one of the purposes of the present study is to determine whether kidney cells of pregnant mice responsible for similar increases are mast cells or non-mast cells. However, since W/WV mice are infertile, we used a model
system instead; namely, castrated male mice treated with estrogen, which has been shown to increase HDC activity (2). As shown in Fig. 3, castration itself caused a slight increase in HDC activity in the kidney, but the subsequent administration of testosterone decreased this activity. On administration of 17-β-estradiol to castrated mice, the HDC activity did not change for 5 days, but after 15 days, it increased greatly. The DOPA decarboxylase (3,4-dihydroxy-L-phenylalanine carboxylase, E.C. 4.1.1.28) activity of the kidneys was not increased by estradiol treatment for 2 weeks: the DOPA decarboxylase activities of the kidneys of control and estradiol-treated mice were 1.52±0.46 (n=5) and 1.74±0.19 (n=3) nmoles/min/g wet weight (means±S.D.), respectively.

Effect of 17-β-estradiol administration on increase of kidney HDC activity of W/WI' mice: Table 1 shows that no HDC activity was detected in the kidneys of untreated W/WI' mice (control) or W/We' mice treated with testosterone, but that it appeared on treatment with 17-β-estradiol. Thus, the increase of HDC activity in the kidney of pregnant mice was due to non-mast cells.

Effect of clomiphene administration on increase of HDC in the kidney induced by 17-β-estradiol: To confirm that the increase in HDC activity by 17-β-estradiol administration is mediated through an estrogen receptor, we tested the effect of administration of clomiphene, an anti-estrogen, on the increase of HDC activity in the kidneys induced by 17-β-estradiol. As shown in Table 2, treatment of castrated mice with 17-β-estradiol caused a remarkable increase in HDC activity in the kidney after two weeks, but treatment with clomiphene alone did not change the activity, and treatment with both 17-β-estradiol and clomiphene blocked the increase of HDC activity caused by 17-β-estradiol only. Thus, clomiphene prevented the increase in HDC activity in mice treated with 17-β-estradiol.

Discussion
In this work, we showed that the increases of HDC activity and histamine content of fetal mice (Fig. 1) and the kidneys of their mothers (Fig. 2) during the later part of gestation were blocked by the administration of α-FMH, a suicide substrate of HDC (7). Since these increases are very large, it is surprising that we observed no abnormalities of the fetuses or mothers on complete blockade of the increase of HDC and histamine with α-FMH. Our results confirm the report of Bartholeyns and Bouclier (18). The blockade of histamine increase by α-FMH was not so complete as that of HDC (Fig. 1). This may be because HDC activity became very low, but not zero, by α-FMH treatment, and histamine once produced should have been stored in granules of mast cells.

As mentioned above, we showed by using W/WI' mice that the increases of HDC activity and histamine content in fetal mice derived from mast cells (17). To determine
Fig. 3. Effects of castration of mice and administration of sex hormones to castrated mice on HDC activity of the kidney. Mice (5 weeks) were castrated and 7 days later treated s.c. with 0.2 mg of 17-β-estradiol on two successive days (on days 7 and 8 after castration) and a further 5 or 15 days later as indicated, they were sacrificed. Some mice were treated with 0.5 mg of testosterone on days 13 and 14 after castration, and on day 15, they were sacrificed. Other groups of mice were killed on day 22 after the start of the experiment. The HDC activity of their kidneys was measured as described in Materials and Methods. Horizontal bars show the S.D. *P<0.005 and **P<0.001 compared to the no treatment group. *P<0.005 compared to the castration group. n=4.

Table 1. Effects of sex hormones on HDC activity in the kidney of W/W^v mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HDC Activity (pmoles/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>n.d. (3)</td>
</tr>
<tr>
<td>17-β-Estradiol</td>
<td>1.62±1.32 (3)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>n.d. (3)</td>
</tr>
</tbody>
</table>

The value is a mean±S.D. for the number of mice shown in parentheses. n.d., not detectable. For details, see text.

Table 2. Effect of clomiphene on increase of HDC activity in mouse kidney induced by 17-β-estradiol

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HDC Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>14.8±3.9 (4)^a</td>
</tr>
<tr>
<td>17-β-Estradiol</td>
<td>74.5±37.1 (6)</td>
</tr>
<tr>
<td>Clomiphene</td>
<td>13.3±8.2 (4)^a</td>
</tr>
<tr>
<td>17-β-Estradiol+Clomiphene</td>
<td>19.8±8.5 (3)^b</td>
</tr>
</tbody>
</table>

Values are mean HDC activities (pmoles/min/g wet weight) of the kidneys of mice treated with 17-β-estradiol, clomiphene, and 17-β-estradiol plus clomiphene as described in Materials and Methods (means±S.D.). Two weeks later, they were killed. Figures in parentheses are numbers of mice treated. Significance of difference between values of the estradiol-treated group and other groups: ^a, P<0.02; ^b, P<0.05. (Student’s t-test)
the type of cells responsible for the increase in kidneys of pregnant mice, we used a model system; namely, we examined the increase in W/W" mice which were treated with estrogen to induce an increase (2), because W/W" mice are infertile. We found that the HDC activity of the kidneys of W/W" mice increased on treatment with 17-β-estradiol, but not testosterone, indicating that the estrogen-sensitive cells are non-mast cells. The increase of HDC activity in the kidney by 17-β-estradiol was less than that found during later pregnancy and varied considerably from experiment to experiment. It is also known that there are great differences in HDC activity between the sexes and among different species of mice (25). However, DOPA decarboxylase activity, which is known to be very high in the kidney (26), was not increased by 17-β-estradiol-treatment. We also showed by blockade of the increase in HDC with clomiphene that the increase is mediated by an estrogen receptor. Although clomiphene has a weak estrogen-agonist activity (27), the HDC activity of the kidney was not increased by treating mice with clomiphene only. In this respect, it is interesting that the ornithine decarboxylase activity is increased by androgen, but not by estrogen, as reported by Rosengren (15) and Seely et al. (28).

Using [3H]-α-FMH to label HDC in vivo, Hammar et al. (29) showed that the kidney cells responding to 17-β-estradiol treatment are present in the cortex, not the medulla, although they did not identify the cells. These data support the results of this paper that the cell type responsible for the increase of HDC activity is a non-mast cell. To examine the location of HDC in the kidney by an immunohistochemical method using anti HDC antibody (30) would be interesting.

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