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TISSUE DISTRIBUTION OF HISTAMINE IN A MUTANT MOUSE DEFICIENT IN MAST CELLS

CLEAR EVIDENCE FOR THE PRESENCE OF NON-MAST-CELL HISTAMINE

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Abstract—The contents of histamine in various tissues of mutant mice deficient in mast cells (W/W^v) and in congenic normal mice (+/+) were determined by high-performance liquid chromatography and were compared. In spite of the absence of mast cells in W/W^v mice, the histamine content of their whole bodies was about 5–10% of that of +/+ mice. The skin, heart and lungs of W/W^v mice contained negligible amounts of histamine (about 2% of that in +/+ mice), but the liver, kidneys and spleen contained appreciable histamine (8–15% of that in +/+ mice), and the brain and stomach contained much histamine (45 and 34%, respectively, of that in +/+ mice). These results indicate the presence of non-mast-cell histamine, especially in the brain and stomach, where it may play important physiological roles.

Most histamine in tissues is stored in mast cells, and the released histamine plays an important role in certain pathophysiological processes [1]. But kinetic studies suggested the presence of another pool of histamine with a more rapid turnover than that in mast cells [2–4], and histochemical studies showed histamine-containing cells that were distinct from mast cells [5, 6]. Histamine liberated from this type of storage is called "non-mast-cell" [7], "induced" [8], or "nascent" [9] histamine and is considered to have specific physiological roles in neural transmission, gastric secretion, and rapid tissue growth [8–13].

There have been many studies on non-mast-cell histamine, but direct evidence for its actual existence is still lacking, and the nature of non-mast cells containing histamine also remains to be clarified. Recently, Kitamura *et al.* found that in W/W^v mice the number of mast cells in the skin is less than 2% of that of congenic normal mice (+/+) and that W/W^v mice have no detectable mast cells in other tissues [14]. Using these mutant mice, we demonstrated that, in spite of the extremely scarce mast cells, the L-histidine decarboxylase activity was not much lower than that in congenic normal mice [15]. This finding strongly suggests the existence of another pool of histamine besides that in mast cells.

In this work, we examined the tissue distribution of non-mast-cell histamine by comparing the contents of histamine in various tissues of W/W^v mice and +/+ mice by an assay method for tissue histamine developed in this laboratory, which has sufficient sensitivity and reproducibility for use in determining small amounts of histamine in tissues.

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MATERIALS AND METHODS

Animals. WBB6F$_1$ (WB-W/+ × C57BL/6-W^v/+) − (W/W^v, +/+) mice were raised in our laboratory using parental stocks originally obtained from the Jackson Laboratory (Bar Harbor, ME, U.S.A.).

Chemicals. Histamine diphosphate and *o*-phthalaldehyde were purchased from Wako Pure Chemical (Osaka, Japan). Other chemicals and reagents were of analytical grade and were used without further purification. Glass double-distilled water was used throughout.

Preparation of tissues for analysis. At 2–4 months after birth, W/W^v mutant mice and their litter-mates (+/+) were decapitated after overnight starvation. Tissues (brain, lungs, heart, stomach, spleen, kidneys, and dorsal skin) were quickly isolated. The tissues were washed with sterile cold saline, blotted with filter paper, weighed on a torsion balance, and promptly homogenized in 5 ml of cold 3% (v/v) perchloric acid by a Polytron homogenizer (Kinematica, Lucerne, Switzerland) operated at the maximal setting for 10 sec in an ice bath. The homogenates were centrifuged at 10,000 *g* for 20 min at 4°. The deproteinized supernatant fractions were applied to a column of Bio Rad AG50 × 8 (4 mm i.d. × 50 mm, Na^+-form). After the tissue extract had passed through, the column was washed with 10 ml of 2 N HCl. Histamine was then eluted with 5 ml of 3 N HCl, and the eluate was evaporated to dryness under reduced pressure.

Analytical procedures. The residue was dissolved in 100–500 μl of 0.05 N HCl, and 50–200 μl of the solution was injected into a stainless steel column packed with TSK-1EX510SP strong cation exchange resin (6 mm i.d. × 150 mm, particle size 5 μm, Toyo Soda, Tokyo, Japan) using an injector valve (VMD-350V, Seishin Pharmaceuticals, Chiba,

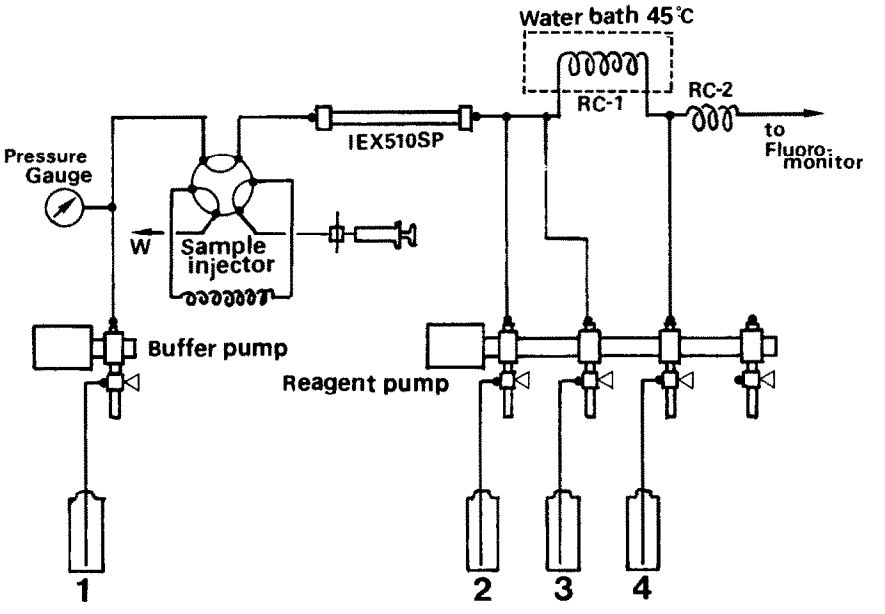

Fig. 1. Flow diagram of the analysis system. Elution buffer and reagents: (1) 0.2 M sodium propionate buffer (pH 4.0) containing 5 mM EDTA-Na_2, 0.5 M NaCl and 20% (v/v) methanol with a flow rate of 0.6 ml/min; (2) 0.125% (w/v) *o*-phthalaldehyde in 50% (v/v) aqueous methanol, with a flow rate of 0.22 ml/min; (3) 1 M NaOH, with a flow rate of 0.20 ml/min; and (4) 4% (v/v) H_2SO_4, with a flow rate of 0.22 ml/min. Separation column: TSK-IEX510SP, 6 mm i.d. × 150 mm, particle size 5 μm, eluted at room temperature (20–25°). Reaction coil; RC-1: PTFE tubing, 0.5 mm i.d. × 5 m in a water bath (45°), and RC-2: PTFE tubing, 0.5 mm i.d. × 1 m. Fluorospectrometer: excitation wavelength, 350 nm; emission wavelength, 450 nm; flow cell, 90 μl.

Japan). The column was eluted with 0.2 M sodium propionate buffer (pH 4.0) containing 5 mM EDTA-Na_2, 0.5 M NaCl, and 20% (v/v) methanol using a constant flow pump (PHD-3.2, Seishin Pharmaceuticals) at a flow rate of 0.6 ml/min. Histamine in the eluate was converted to a fluorescent product by the *o*-phthalaldehyde condensation method [16, 17] using a continuous flow reaction system (Fig. 1). Three plungers of a quadruple plunger pump (PSU-2.5T, Seishin Pharmaceuticals) served independently to deliver reagents (the remaining plunger was not used here), and polytetrafluoroethylene (PTFE) tubing, 0.5 mm i.d. × 5 m (reaction-coil 1), 0.5 mm i.d. × 1 m (reaction-coil 2), was used for mixing the eluate with the reagents. The eluate was mixed first with 0.125% (w/v) *o*-phthalaldehyde in 50% (v/v) aqueous methanol (0.22 ml/min) and then with 1 M NaOH (0.20 ml/min). After mixing in reaction-coil 1 at 45° by placing the coil in a water bath (Ministat, Huber, Elgersweier, West Germany), 4% (v/v) H_2SO_4 was added (0.22 ml/min) and mixed in reaction-coil 2. The fluorescent intensity was measured at 450 nm (excitation at 350 nm) in a spectrofluorometer (650-10, Hitachi, Tokyo, Japan) equipped with a 90 μl square flow cell and a recorder (056, Hitachi).

RESULTS AND DISCUSSION

Determination of tissue histamine. Histamine and other substances reacting with *o*-phthalaldehyde, such as ammonia and spermidine, could be separated within 30 min, as shown in Fig. 2. Histidine and spermine, which also reacted with *o*-phthalaldehyde, were removed by the sample clean-up procedure by AG50 column chromatography. Figure 3 shows a typical elution pattern of histamine from the brain of a +/+ mouse. The peaks of ammonia and sper-

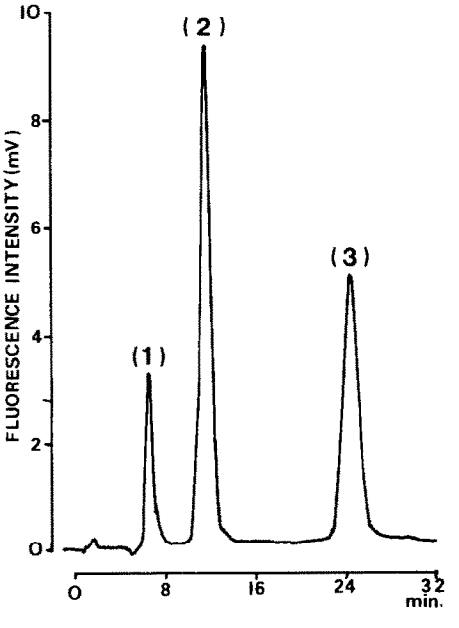

Fig. 2. Chromatogram of standard sample. Key: (1) ammonia, 20 nmoles; (2) histamine, 50 pmoles; and (3) spermidine, 5 nmoles.

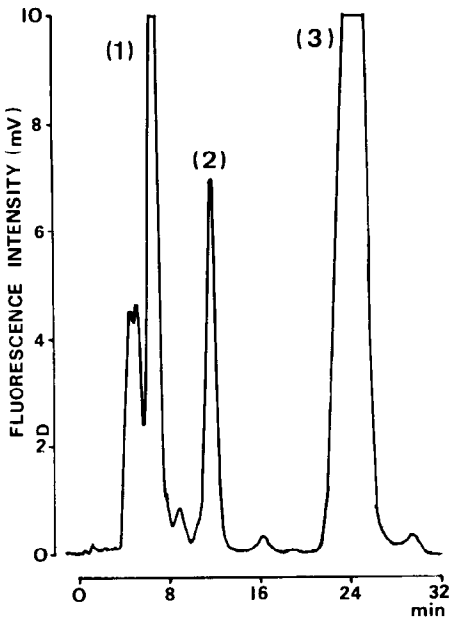

Fig. 3. Chromatogram of mouse brain sample. Histamine in the deproteinized supernatant fraction of the brain tissue of a +/+ mouse was purified by AG50 column chromatography, and a sample corresponding to 82 mg of the brain tissue was injected. Key: (1) ammonia; (2) histamine, 36.9 pmoles; and (3) spermidine.

midine are well separated and no interfering or overlapping peaks are observed. The amount of histamine was linearly related to the fluorescence intensity (expressed as peak height) with 5 pmoles to 10 nmoles of histamine (Fig. 4). The reproducibility, calculated from separate determinations on five aliquots of pooled brain homogenate, and the over-all recovery rate of histamine added to the same brain homogenate are shown in Table 1. The coefficient of variation in the assay was within 2%, and the recovery rate throughout the whole procedure was 94%.

Histamine contents of the tissues of mice. We measured the histamine contents of the whole bodies of W/W^v and +/+ mice. Measurement on the decapitated whole bodies of three W/W^v mice and three +/+ mice showed that the histamine contents were 4.3 ± 1.9 nmoles and 56.3 ± 9.7 nmoles/g of wet tissue (mean ± S.D.) respectively. These values are in good agreement with those for whole mice reported previously [15]. Thus, in spite of the absence of mast cells, histamine was present in the whole bodies of W/W^v mice at about 5–10% the level in +/+ mice. This value of 5–10% was considered to represent the content of non-mast-cell histamine in the whole body of mice.

Table 2 summarizes the histamine contents of various tissues of +/+ and W/W^v mice. The histamine contents of the stomach, skin, heart, lungs, spleen, kidneys, brain and liver of normal mice were 99.5 ± 9.9, 91.6 ± 15.3, 5.58 ± 0.39, 4.86 ± 1.11, 3.11 ± 0.58, 0.81 ± 0.07, 0.48 ± 0.06 and 0.40 ± 0.04 nmoles/g of wet tissue (mean ± S.E.M.) respectively. These results are in good agreement with data on the histamine contents of various tissues of mice reported by Anton and Sayre [18]. The histamine contents in these respective tissues in W/W^v mice were 33.9 ± 4.0, 1.83 ± 0.38, 0.09 ± 0.01, 0.10 ± 0.02, 0.26 ± 0.07, 0.11 ± 0.02, 0.22 ± 0.02 and 0.06 ± 0.02 nmoles/g of wet tissue (mean ± S.E.M.).

The histamine contents of these tissues were also determined by the dansylation method [19]. The values for +/+ mouse tissue and for whole bodies of both normal and mutant mice were comparable to the present results, although values for all tissues of W/W^v mice except the stomach were somewhat higher than the present results, owing to overlapping of unknown interfering materials with the dansyl-histamine peak.

Distribution of non-mast-cell histamine. The his-

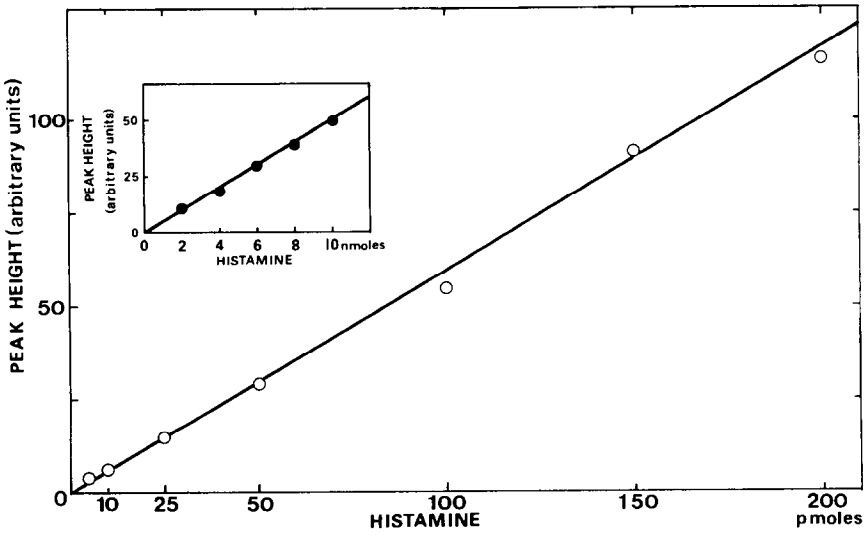

Fig. 4. Standard calibration curve for the assay. Two hundred microliters of histamine solution in 0.05 N HCl was injected.

Table 1. Reproducibility of the assay system and recovery of tissue histamine*

	Fluorescence intensity (peak height, arbitrary units)		
	Histamine†	Brain homogenate‡	Brain homogenate + histamine§
1	54.0	54.5	106.5
2	54.0	55.2	107.3
3	55.3	56.0	108.2
4	55.7	57.2	108.2
5	56.1	57.4	110.0
Mean ± S.D.	55.0 ± 0.82	56.1 ± 1.12	108.0 ± 1.17

* The recovery rate was calculated as $[(108.0 - 56.1)/55.0] \times 100 = 94.4\%$. The coefficient of variation in the assay was within 2%.

† One hundred pmoles of histamine was dissolved in 250 μl of 0.05 N HCl and 200 μl of the solution was injected into the IEX510SP column.

‡ Eight brains of +/+ mice (2,880 mg) were homogenized with 60 ml of 3% perchloric acid and centrifuged at 10,000 *g* for 20 min at 4°. Then, 5 ml of the supernatant fraction was purified by AG50 column chromatography. The histamine fraction was evaporated and dissolved in 250 μl of 0.05 N HCl, and 200 μl of the solution was injected into the IEX510SP column.

§ One hundred pmoles of histamine was added to 5 ml of the deproteinized supernatant fraction and was purified and assayed as described in the ‡ footnote.

tamine contents of the brain and stomach of W/W^v mice were 45 and 34%, respectively, of those of normal mice; the histamine contents of their liver, kidneys and spleen were 15, 14 and 8%, respectively, of those of normal mice; and the histamine contents of their lungs, skin and heart were only 2% of those of normal mice (Table 2). These percentages were considered to be non-mast-cell histamine contents in these tissues of mice, the highest value being that in brain. Cerebral histamine has been suggested to be a putative neurotransmitter [11, 20, 21], although this is still controversial. Biochemical [22–25] and morphological [26–30] studies have shown that cerebral histamine is stored in both neurons and mast cells, and that mast cells in the brain are closely associated with the vascular walls [24, 30], and have a high histamine content and low L-histidine decarboxylase activity [22, 23]. Garbarg *et al.* [31] showed that after administration of α-fluoromethylhistidine, a highly specific irreversible inhibitor of L-histidine decarboxylase, the histamine levels in the hippocampus, medulla oblongata, hypothalamus, cerebral cortex, and striatum decreased only 20, 40, 60, 60, and 70%, respectively, while the L-histidine decarboxylase activity was almost completely lost. The histamine that decreased (about half the total histamine in brain) had a very rapid turnover rate and was considered to be non-mast-cell histamine. Their results were in good agreement with ours, namely, that non-mast-cell histamine and mast-cell histamine are present in almost equal amounts in whole brain.

Gastric histamine is also thought to be stored in both mast cells and enterochromaffin-like cells, and histamine in the latter has been suggested to be a mediator of gastric secretion [12]. The present results show that the stomach of W/W^v mice contains a significant amount of histamine in spite of the absence of mast cells. Furthermore, we recently demonstrated that the distribution and number of enterochromaffin-like cells in the fundic mucosa of W/W^v mice were not significantly different from those in the normal mice, and that the glandular stomach of W/W^v mice contained 100 times more histamine than the forestomach, whereas in normal mice the two regions contain similar amounts of histamine [32]. These results strongly suggest that the non-mast-cell histamine of the mice may be important in gastric secretion.

The skin, heart and lungs of W/W^v mice contained only negligible amounts of histamine (about 2% of that in normal mice). Histamine in these tissues is considered to be essentially all of mast cell origin.

Table 2. Histamine contents in various tissues of W/W^v and +/+ mice*

Tissue	W/W^v [A]	N	+/+ [B]	N	([A]/[B]) × 100 (%)
Brain	0.22 ± 0.02	5	0.48 ± 0.06	6	44.7
Stomach	33.9 ± 4.0	4	99.5 ± 9.9	5	34.1
Liver	0.06 ± 0.02	6	0.40 ± 0.04	6	14.8
Kidney	0.11 ± 0.02	7	0.81 ± 0.07	4	13.5
Spleen	0.26 ± 0.07	6	3.10 ± 0.58	5	8.4
Lung	0.10 ± 0.02	7	4.86 ± 1.11	6	2.1
Skin	1.83 ± 0.38	5	91.6 ± 15.3	5	2.0
Heart	0.09 ± 0.01	5	5.58 ± 0.39	5	1.7

* Values are averages ± S.E.M. (nmoles/g of wet tissue); N is the number of animals assayed.

In the liver, kidneys and spleen of W/W^v mice, histamine amounted to 8–15% of that in normal mice. These tissues might have non-mast-cell histamine pools and, if so, the physiological roles of non-mast-cell histamine in these tissues seem very interesting.

Schrader *et al.* [33] have recently reported development of mast-cell-like histamine-containing cells *in vitro* from the bone marrow of (C57BL/6 × DBA/2)F_1-W^f/W^f mice. Since the defect in hematopoietic cells of *W* mutant mice is not detectable with *in vitro* systems [34–36], the result of Schrader *et al.* does not necessarily mean the presence of such histamine-containing bone-marrow-derived cells in tissues of *W* mutant mice *in vivo*. However, the *in vitro* method may be useful for further understanding of the mechanism of mast-cell depletion in *W* mutant mice.

In this paper, we present clear evidence for the existence of non-mast-cell histamine and information on its distribution. W/W^v mice will be useful for studies on the roles of non-mast-cell histamine as well as mast-cell histamine.

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