Biochemical Pharmacology, Vol. 32, No. 1, pp. 59–63, 1983.
Printed in Great Britain.

0006-2952/83/010059-05 $03.00/0

HISTIDINE UPTAKE BY ISOLATED RAT PERITONEAL MAST CELLS

EFFECT OF INHIBITION OF HISTIDINE DECARBOXYLASE BY α-FLUOROMETHYLHISTIDINE*

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(*Received* 1 *February* 1982; *accepted* 17 *May* 1982)

Abstract—Preincubation with (*S*)-α-fluoromethylhistidine, an irreversible inhibitor of histidine decarboxylase, was found to markedly reduce, but not eliminate, the uptake of [^{3}H]histidine by rat peritoneal mast cells. The V_{max} for histidine transport for cells in which decarboxylation of histidine had been completely inhibited was 11.9 pmoles per min per 10^6 cells, compared to a V_{max} of 18.9 pmoles per min per 10^6 cells in the presence of active mast cell histidine decarboxylase. The K_m of uptake was 139 μM in the presence of α-fluoromethylhistidine, several times higher than the K_m of 44.0 μM in the uninhibited cell. α-Fluoromethylhistidine did not inhibit mast cell uptake of phenylalanine, a competitive inhibitor of histidine uptake but not a substrate for histidine decarboxylase; nor did it inhibit the uptake of histidine by non-mast cells, which lack histidine decarboxylase. Levels of intracellular [^{3}H]histidine in mast cells were similar in the presence and absence of the decarboxylase inhibitor. Based on these observations, we propose that intracellular decarboxylation of histidine in the mast cell serves to specifically enhance the uptake of histidine by the relatively non-specific amino acid transporter present in the plasma membrane of the cell.

Mast cells are the primary storage site for the vasoactive amine, histamine. Schayer [1] and Cabut and Haegermark [2] have demonstrated the uptake and decarboxylation of histidine by peritoneal mast cells. Histidine decarboxylase activity has been measured in cell homogenates [3, 4]; this activity is considerably lower than in intact cells [5, 6] and, thus, considerable caution is required in interpretation of studies of enzyme activity in disrupted cell preparations. However, Yamada *et al.* [7] have found recently that the use of a protease inhibitor greatly increases the decarboxylase activity recovered from disrupted mast cells.

We have reported previously on the ability of isolated, intact rat peritoneal mast cells to take up histidine, efficiently convert the internalized histidine to histamine, and store the nascent amine in the granules specific for this cell type [8]. Consideration of (a) the apparent kinetic constants determined for histidine uptake, (b) the values previously determined for histamine uptake, and (c) estimates of histidine and histamine concentrations in plasma led us to propose that, under usually prevailing circumstances *in vivo*, circulating histidine is the predominant source of mast cell histamine. Based on our ability to substantially block histidine uptake by mast cells with L-phenylalanine and DL-B-2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (BCH), we suggested that the transport system largely responsible for moving histidine into the cell is similar to the L system defined in Christensen's laboratory [9–11] for other cell types.

We now report the effects of α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, on mast cell histidine transport.

MATERIALS AND METHODS

Percoll was purchased from Pharmacia Fine Chemicals, Piscataway, NJ, (*S*)-α-fluoromethylhistidine was supplied by Dr. J. Kollinitsch (Merck, Sharp & Dohme Research Laboratories, Rahway, NJ). L-[Side chain-^{3}H]Phenylalanine (24 Ci/mmole) was purchased from ICN Pharmaceuticals, Inc., Irvine, CA. L-[2,5-^{3}H]Histidine (42 Ci/mmole) was purchased from Amersham/Searle, Arlington Heights, IL. Other materials were obtained from previously specified sources [8].

Mast cells were collected from the peritoneal cavities of male Sprague–Dawley rats (Hilltop Laboratory Animals, Scottdale, PA, or Charles River Breeding Laboratories, North Wilmington, MA) as previously described [12]. Mast cells were separated from non-mast cells by either the albumin method [12] or by a modification of the Percoll isolation method of Enerbäck and Svensson [13]. In this latter procedure, peritoneal lavages from four to eight rats were combined and spun for 8 min at 150 *g*, and the pellet was resuspended in 4–5 ml BSSA‡ [12]. The resulting suspension was carefully layered over 10 ml of buffered Percoll (7 ml Percoll, 1 ml 10x salts solution, 0.14 ml albumin, pH adjusted to 7.2 with 0.1 M NaH_2PO_4, adjusted to a final volume at 10.0 ml

* Supported by Grant HL25402 from the National Heart, Lung and Blood Institute.

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‡ Abbreviations: BSSA, balanced salt solution with albumin; HEPES, *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; and OPT, *o*-phthalaldehyde.

with H_2O; the 10x salt solution contained 1.54 M NaCl, 27 mM KCl and 6.8 mM $CaCl_2$). Following centrifugation at 225 *g* for 15 min, the mast cells, which formed a pellet, and the non-mast cells, which remained at the interface, were separately collected and each washed several times with BSSA. The cells were resuspended in sufficient BSSA to give a concentration of approximately 10×10^6 cells/ml. Cell numbers were determined with a Neubauer hemocytometer. The mast cell fraction isolated by either method contained 85–95% mast cells, whereas the non-mast cell fraction was composed largely of macrophages, 2–3% mast cells and a similar low percentage of eosinophils.

The concentration dependence of the inhibition of histidine uptake by α-fluoromethylhistidine was measured using aliquots of 0.5×10^6 cells. The cells were preincubated with volume of a stock solution of α-fluoromethylhistidine (10 mM in H_2O) sufficient to give the desired concentration of inhibitor. The cells were preincubated for 10 min at 37°, centrifuged (11,500 *g* for 0.5 min), and washed once with 250 μl of BSSA buffer. [^{3}H]Histidine uptake was then measured for 1 min according to the protocol previously described [8]. The standard final histidine concentration was 1.6 μM. In other experiments, aliquots of 100 μl containing 10^6 suspended mast cells were preincubated with 2×10^{-4} M α-fluoromethylhistidine and then washed as described above; the radioactivity taken up after varying times of incubation was separated into [^{3}H]histidine and [^{3}H]histamine fractions by TLC as previously described [8].

Studies of the effect of α-fluoromethylhistidine on mast cell phenylalanine uptake were carried out in similar fashion. Fifty-microliter aliquots of cell suspension containing 0.5×10^6 mast cells or non-mast cells were preincubated with 1 μl of 10 mM α-fluoromethylhistidine for 10 min at 37°. Following a brief centrifugation and wash, as described above, the cells were incubated with 5 μCi of [^{3}H]phenylalanine for 1 min according to the standard protocol for measurement of histidine uptake. The concentration of phenylalanine was 2.8 μM.

To examine [^{3}H]histidine distribution within the mast cell after preincubation with α-fluoromethylhistidine, the cells were incubated in [^{3}H]histidine as described above. Washed cell pellets were then resuspended in 0.5 ml BSSA buffer and disrupted by sonication in a Sonogen bath sonicator (Branson Instruments, Inc., Stamford, CT) for a period of time, 20–40 sec, sufficient to give 90–100% cell lysis. An aliquot of total sonicate was removed, and one-half volume of 7.5% trichloroacetic acid (TCA) was added. The remainder of the sonicate was vortexed, and the radioactivity and the histamine content of the TCA supernatant fraction were determined. The remainder of the total sonicate was carefully layered over 1.5 ml of Percoll with added salts (9 ml Percoll, 1 ml 10x HEPES-salts and 0.14 ml albumin; the 10x HEPES-salts were prepared by adjusting a solution of 0.1 M HEPES, 1.45 M NaCl, 27 mM KCl and 6.8 mM $CaCl_2$ to pH 4). The samples were spun for 20 min at 27,000 *g* in an angle head centrifuge, and the granule fraction with intact membranes [14] was removed, washed with BSSA buffer, and resuspended in a small volume of the same buffer. One-half volume of 7.5% TCA was added, and the sample was vortexed and centrifuged to remove the TCA precipitate. Radioactivity and histamine content of the TCA supernatant fraction were determined. The amount of radioactive histidine in the intact granule fraction was corrected for granule membrane breakage based on the histamine content of the isolated granules relative to that in the total sonicate, assuming all the histamine was originally in the granules. The chemical assay for histamine with OPT was performed as previously described [15].

RESULTS

Preliminary experiments were performed to determine the effect of varying the concentration of α-fluoromethylhistidine on the uptake of radioactivity by mast cells exposed to [^{3}H]histidine in the medium. A sigmoidal concentration–inhibition curve with a 50% inhibition at 2×10^{-6} M was found, as shown in Fig. 1. The kinetics of uptake of [^{3}H]histidine were studied after preincubation with 2×10^{-4} M α-fluoromethylhistidine. The results, presented in Fig. 2, indicate that in uninhibited cells uptake of radioactivity continued at a substantial rate for the 30-min duration of the experiment. Very little radioactivity was accumulated by mast cells pretreated with α-fluoromethylhistidine; what radioactivity was taken into the cell was incorporated in the first 5 min with very little increase in uptake thereafter.

When intracellular [^{3}H]histamine that was a result of decarboxylation of [^{3}H]histidine was separated from intracellular [^{3}H]histidine, the results presented in Fig. 3 were obtained. While the amounts of [^{3}H]histidine were quite comparable at all but the earliest time interval in the control cells and in those treated with α-fluoromethylhistidine, [^{3}H]histamine was reduced markedly in the cells treated with the inhibitor. The measured ratio of the [^{3}H]histamine radioactivity of inhibited cells at 30 min to that of

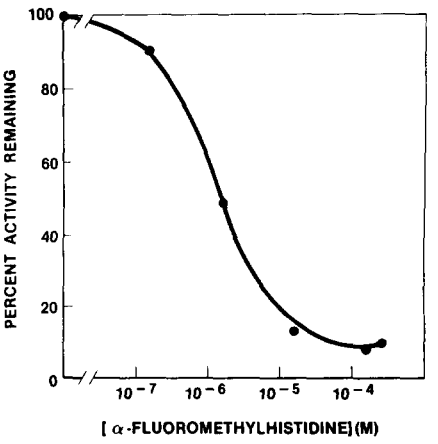

Fig. 1. Inhibition of [^{3}H]histidine uptake by α-fluoromethylhistidine; effect of concentration. Mast cells were incubated in various concentrations of α-fluoromethylhistidine for 10 min at 37°, washed in BSSA and exposed to [^{3}H]histidine (1.6 μM) for 1 min at 37° and then washed three times in BSSA; TCA extractable radioactivity was measured. Each point is the mean of two determinations.

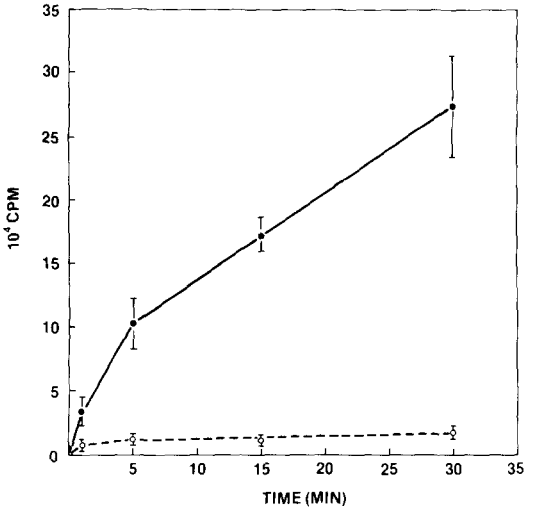

Fig. 2. Inhibition of [^{3}H]histidine uptake by α-fluoromethylhistidine; kinetics of uptake. Mast cells were preincubated in BSSA for 10 min at 57° with 2×10^{-4} M α-fluoromethylhistidine (O– – –O) or without the inhibitor (●——●) and then exposed to [^{3}H]histidine (1.6 μM) for varying times and washed; then cell radioactivity was measured. Points are the means ± S.E. of the results in three separate experiments.

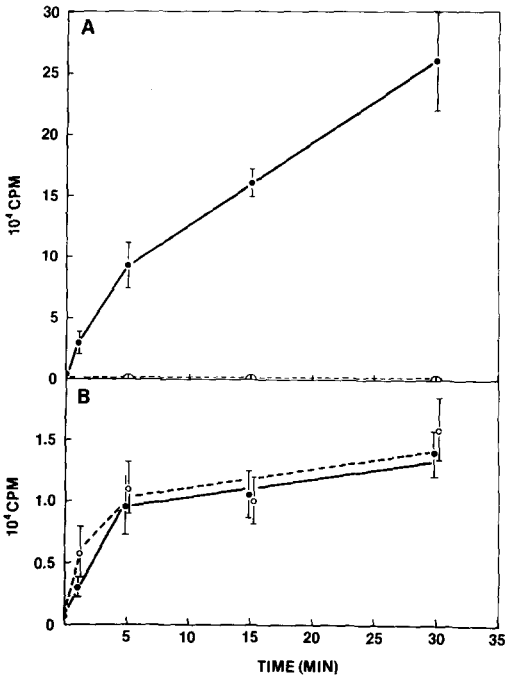

Fig. 3. Effect of α-fluoromethylhistidine on mast cell levels of [^{3}H]histidine and [^{3}H]histamine after exposure to [^{3}H]histidine. Cells were treated as described for Fig. 2, but the radioactivity extracted from the cells was separated into histidine and histamine fractions by TLC and then measured. (A) [^{3}H]Histamine. (B) [^{3}H]Histidine. Key: control cells (●——●); and cells treated with inhibitor (O– – –O). Points are the means ± S.E. of the results in three separate experiments.

control cells at 30 min was 3/1000. Histidine uptake by non-mast cells was inhibited to only a minor extent by α-fluoromethylhistidine (Table 1), and the uptake of phenylalanine, a competitive inhibitor of histidine uptake by mast cells, was increased slightly, but not to a level of statistical significance (Table 1).

In an attempt to determine the kinetic parameters for histidine uptake by mast cells unperturbed by subsequent decarboxylation, the velocity of uptake of [^{3}H]histidine by cells treated with α-fluoromethylhistidine was measured at various concentrations of histidine in the medium. The results were plotted according to the method of Augustinsson (Fig. 4), and the parameters were determined by linear regression analysis. V_{max} was found to be 11.9 pmoles per min per 10^6 cells and K_m, 139 μM.

To determine how much, if any, of the histidine that enters the cell is sequestered in the specific granules, mast cells were preincubated with α-fluo-

Table 1. Inhibition of amino acid uptake in mast cell and non-mast cell fractions by α-fluoromethylhistidine*

Uptake system		Counts/min in cells†	
Cells	Amino acid	Control	α-Fluoromethylhistidine (2×10^{-4} M)
Mast cells	[^{3}H]Histidine	5800 ± 590 (793)‡	440 ± 10§ (184)
Mast cells	[^{3}H]Phenylalanine	1080 ± 60 (303)	1260 ± 120‖ (294)
Non-mast cells	[^{3}H]Histidine	1090 ± 180 (762)	990 ± 30‖ (304)

* Mast cell and non-mast cell fractions were isolated and assayed as described in Materials and Methods.

† Counts/min taken up by 0.5×10^6 cells in 1 min at 37°. Cells were exposed to 2×10^{-4} M α-fluoromethylhistidine for 10 min at 37°. Values are the means ± S.E. of these determinations, corrected for uptake at 0°.

‡ The values in parentheses are the cpm determined in cells incubated with radioactive amino acid at 0° for 1 min.

§ Difference between control and treated means is significant by Student's t-test at $P < 0.001$.

‖ Difference between control and treated means is not significant by Student's t-test at $P = 0.05$.

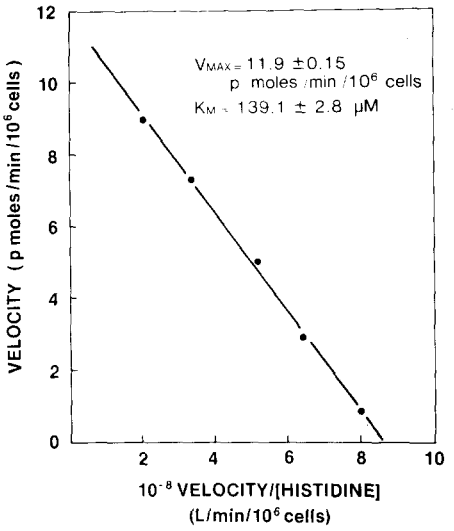

Fig. 4. Augustinsson plot for [^{3}H]histidine uptake by mast cells inhibited with α-fluoromethylhistidine. Mast cells were pretreated with 2×10^{-4} M α-fluoromethylhistidine for 10 min at 37° and then exposed to various concentrations of histidine; the radioactivity taken up by the cells in 1 min was measured. The points are calculated from means of three determinations of the velocity at each of five concentrations of histidine. The solid line is the best fit determined by linear least squares. The values for V_{max}, K_m and their standard errors were calculated by linear regression analysis.

romethylhistidine and then incubated for 15 min with [^{3}H]histidine. The cells were disrupted by sonication, and the granule fraction with intact membranes was isolated by centrifugation of the sonicate on a Percoll gradient. Since essentially no histamine is formed in cells inhibited with α-fluoromethylhistidine, determination of radioactivity in the granule provides a direct measure of the amount of [^{3}H]histidine transferred into the granules. Determination of [^{3}H]histidine in the granules, when corrected for the loss from granule membrane breakage and release of [^{3}H]histidine, yielded a value of 12.6 ± 2.7% (mean of three determinations ± S.E.) of the total cell histidine.

DISCUSSION

We have described previously the characteristics of the uptake and decarboxylation of histidine in peritoneal mast cells and of the transport of the nascent histamine into the mast cell granule [8]. To further analyze the intracellular pathway of histamine formation and storage, we undertook experiments in which the enzymatic conversion of histidine to histamine was blocked. (*S*)-α-Fluoromethylhistidine has been shown previously to be an effective irreversible suicide inhibitor of histidine decarboxylase from sources other than mast cells *in vivo* and *in vitro* [16, 17]. We were able to confirm that this agent prevented the conversion of histidine to histamine by mast cells. We were initially surprised to find considerable inhibition of uptake of [^{3}H]histidine radioactivity by mast cells. It turned out, however, that a similar observation had been made by Cabut and Haegermark [2] using another decarboxylase inhibitor, NSD 1055.

We considered two alternative possibilities that could account for the inhibition of histidine uptake: either α-fluoromethylhistidine was acting directly on the transport system, or inhibition of the conversion of histidine to histamine in the mast cell markedly inhibited histidine uptake indirectly. Results of two tests of the alternatives favor the latter possibility. Histidine transport by non-mast cells that lack histidine decarboxylation was not inhibited significantly by α-fluoromethylhistidine (Table 1), and the uptake by mast cells of phenylalanine, which competes for the transport system responsible for the bulk of histidine uptake [8] and is, apparently, taken up to some considerable extent by the same transport system [18], was not inhibited by α-fluoromethylhistidine (Table 1).

Based on kinetic studies of the [^{3}H]histidine and [^{3}H]histamine levels in mast cells and specifically in mast cell granules [8], we previously proposed that histidine is converted to histamine in the cytoplasm and that the histamine transferred into the granule. If this is correct, then the decrease in V_{max} for histidine transport with elimination of decarboxylase activity and the similar steady-state levels of intracellular [^{3}H]histidine in the presence and absence of decarboxylase activity (Fig. 3B) can be explained by a kinetic model in which the transport of histidine out of the cell is slowed by conversion of intracellular histidine to histamine during the approach to steady-state levels of intracellular histidine. At near steady-state levels, when the rate of back transport approaches that of transport into the cell, net accumulation of radioactivity will be determined by the rate of conversion of histidine to histamine. The increase in the apparent K_m for transport, when the decarboxylase is inhibited by α-fluoromethylhistidine, is explicable, without invoking any direct effects of α-fluoromethylhistidine on the actual transport system, on the basis of a greater internal histidine concentration at early times (Fig. 3B), since the apparent K_m for transport is equal to $K_m \times$ [histidine inside]/[histidine outside].

Comparison of the limited accumulation (12%), of cell histidine in the granules with the virtually complete sequestration of newly formed histamine in the granule after 15 min of continuous exposure to [^{3}H]histidine [8] indicates the likelihood of a substantial preference for histamine over histidine in transport across the granule membrane. This preference is in contrast to that of the plasma membrane for transporting histidine. Such a conclusion must be somewhat tempered by the lack of any reliable information on the actual concentrations of free amine or amino acid in any compartment but the external medium.

The mechanism for efficient sequestration of histamine in mast cell secretory granules, according to our interpretation, consists then of several components: (1) a banal amino acid transport system in the plasma membrane, (2) specific stimulation of histidine uptake by the action of histidine decarboxylase, (3) conversion of histidine to histamine by the decarboxylase and (4) selective movement of the

nascent histamine across the granule membrane to its storage site in the granule.

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