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HISTAMINE SYNTHESIS IN INTACT AND DISRUPTED RAT MAST CELLS

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Abstract—Histamine production by purified intact rat peritoneal mast cells, as measured by formation of [β-^{3}H]histamine from [β-^{3}H]L-histidine or by release of $^{14}CO_2$ from ^{14}C-carboxyl-labeled histidine, was ten to thirty times greater than that of disrupted cells or soluble extracts of these cells. Loss of activity was evident whether cells were disrupted by sonification, freezing and thawing, or lysis, both in the absence and presence of inhibitors of proteolytic enzymes and agents known to preserve enzyme activity. Studies with decarboxylase inhibitors indicated that a specific histidine decarboxylase was responsible for histamine formation in both the intact cells and cell extracts. In the presence of subsaturating concentrations of histidine, various histidine analogs and glutamine inhibited histidine uptake and histamine formation in intact mast cells but did not inhibit synthesis in cell extracts. These data indicate that, at physiological concentrations of histidine, blockade of histidine transport (through system N) may limit histamine synthesis in the intact cell and that measurement of histidine decarboxylase activity in tissue homogenates or cell extracts may not reflect actual histidine decarboxylase activity *in vivo*.

Histamine is found in mammalian tissues in amounts ranging from less than 0.1 μg/g in blood to over 50 μg/g in gastric mucosa and lung and over 1 mg/g in mastocytomas [1, 2]. The enzyme responsible for histamine synthesis, histidine decarboxylase (EC 4.1.1.22), has been identified in extracts of many tissues [3–8]. This enzyme is specific for histidine and is inhibited by α-methylhistidine but not, except in high doses, by α-methylDOPA [4, 7].§ These characteristics distinguish it from the nonspecific aromatic amino acid decarboxylase (DOPA decarboxylase; EC 4.1.1.26), which has a low affinity (K_m = 10^{-2} M) for histidine and is inhibited by α-methylDOPA but not by α-methylhistidine [4]. Histidine decarboxylase activity in adult rat tissues other than stomach, however, is low and is insufficient to account for the high histamine levels, unless histamine turnover occurs at an extremely slow rate [9]. We report here that the rate of histamine synthesis, in the presence of physiological and saturating concentrations of histidine, is much greater in the intact cell than in extracts of these cells and that histamine synthesis can be reduced by blocking histidine uptake into the mast cell.

MATERIALS AND METHODS

Materials. Drugs and reagents were obtained from the following sources: trypsin inhibitor (soybean, type 1-S), phenylmethylsulfonyl fluoride, and pepstatin-A, Sigma Chemical Co., St. Louis, MO; leupeptin, antipain, and chymostatin, Peninsula Laboratories, San Carlos, CA; Ficoll 400, Pharmacia Fine Chemicals, Uppsala, Sweden; Triton X-100, Research Products Int., Elk Grove Village, IL; Hyamine hydroxide, 1 M, Amersham/Searle Corp., Arlington Heights, IL; bovine plasma albumin and HEPES, 1 M, buffer, Grand Island Biological Co., Grand Island, NY; polyethylene glycol 400 and disodium ethylene diamine tetraacetate (EDTA), Fisher Scientific Co., Fair Lawn, NJ; dithiothreitol (Cleland's reagent) and L-histidine monohydrochloride monohydrate, Schwarz/Mann, Orangeburg, NY; glutamine, α-methylaminoisobutyric acid and pyridoxal-5-phosphate, Sigma Chemical Co.; L-tryptophan, CalBiochem, La Jolla, CA; β-thienylalanine, Z. D. Gilman Inc., Washington, DC; α-methyl-L-histidine and α-methyl-L-DOPA were gifts from Merck, Sharp & Dohme Research Laboratories, Rahway, NJ; Brocresine (NSD 1055) was a gift from Lederle Laboratories Division, Pearl River, NY. The fluorinated derivatives of histidine (hydrochloride salts) were synthesized for us by Dr. K. L. Kirk, Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, MD.

Radioactive materials were obtained from the New England Nuclear Corp. Boston, MA, and the Amersham/Searle Corp. Reagents and media used for cell separation were as described in our previous publications. Additional reagents used in this study included Hanks' medium, HEM Research Inc., Rockville, MD. All other buffers were prepared with analytical grade chemicals.

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§ Abbreviations: DOPA, 3,4-dihydroxyphenylalanine; and HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

Collection and purification of cells. Studies were conducted with crude cell suspensions as well as with cells prepared by elutriation or Ficoll density gradient separation. Male Sprague–Dawley rats (180–300 g) were decapitated and 10 ml of Hanks' balanced salt solution, which contained 50 mM HEPES and 0.1% bovine serum albumin, was injected into the abdominal cavity. The abdomen was then massaged for 90 sec, the abdominal wall was opened by midline incision, and the fluid was removed by pipette. The peritoneal cells were recovered by centrifugation, washed once with 10 ml of ice-cold Hanks'–HEPES–albumin solution, and resuspended in 10 ml of the Hanks' solution. All centrifugations were at 100 *g* for 10 min at room temperature.

In studies of the distribution of histamine and histidine decarboxylase in the different cell fractions, the cell suspension was loaded into an elutriator rotor (Beckman Instruments, Spinco Division, Fullerton, CA). Medium was passed through the elutriator at a constant flow rate [10, 11] of 20 ml/min, and nine fractions (100 ml) were collected at the following rotor speeds: 2200, 2000, 1800, 1600, 1500, 1400, 1300, 1200, and 1100 rpm. The final fraction (No. 10) consisted of the residual material remaining in the separation chamber. Cell counts and size analysis were performed using a Particle Data Counter [10]. The viability of cells was assessed by trypan blue exclusion, by light and electron microscopy, and by their ability to degranulate in response to Compound 48/80 [10].

The effects of various agents and treatments on histidine decarboxylase activity and histidine transport were studied with peritoneal cell suspensions and mast cells purified by a single step purification procedure, based upon the techniques of several authors [12–14]. Peritoneal cells ($\sim 20 \times 10^{-6}$) were resuspended in 20 ml of the Hanks'–HEPES–albumin solution, and four 5-ml aliquots of the suspension were placed on top of two layers of Ficoll solution placed in four centrifuge tubes (15 ml volume polycarbonate tubes). Each tube contained 2.5 ml of a 40% (w/v) Ficoll solution beneath 1.5 ml of a 30% (w/v) Ficoll solution (in Hanks'–HEPES–albumin). The tubes were centrifuged at 250 *g* for 15 min. The upper aqueous layer and the thick layer of peritoneal cells (with a few mast cells) at the interface of the aqueous 30% Ficoll layers were removed and the inside of the upper part of the tube was wiped clean with a cotton swab. Mast cells were located at the interface of the two Ficoll layers and were redispersed in 10 ml of Hanks'–HEPES–albumin solution. The cells were then deposited by centrifugation at 200 *g* for 10 min. These cells (yield 4–6 $\times 10^6$ cells) contained 87 ± 2% (range 70–98%, N = 20) mast cells.

Other preparations studied included partially purified fractions of rat stomach enterochromaffin-like (ECL) cells [11] and a soluble extract of rat gastric mucosa (soluble histidine decarboxylase) [15]. The ECL cells were purified by procedures described by Soll *et al.* [11] and contained from 8 to 12% ECL cells.

Assay procedures. For the assay of histamine and enzyme activities, 1-ml samples of the cell suspensions were centrifuged at 100 *g* for 10 min and the supernatant fluid was removed by aspiration. The pelleted cells were dispersed in Hanks' salt solution containing 10 mM HEPES buffer (pH 7.4) (0.5×10^6 cells/200 μl) or other media as indicated in the text. Where noted, cells were disrupted by sonification (Kontes Ultrasonicator, maximum setting) for 10 sec, freezing and thawing three times (on Dry Ice), or by lysing with water or Triton X-100 (4%, w/v). Histidine decarboxylase activities were assayed in samples of the intact and disrupted cell preparations by a microprocedure in which $^{14}CO_2$ release from ^{14}C-carboxyl-labeled L-amino acid was measured [16]. The standard reaction mixture contained the cell preparation (5×10^4 cells, unless indicated otherwise), 10 μM pyridoxal phosphate, 20 nCi ^{14}C-carboxyl-labeled L-histidine, unlabeled amino acid in various concentrations, and medium to a final volume of 40 μl. The samples were incubated for 30 min, and the $^{14}CO_2$ was trapped in Hyamine hydroxide [16]. The specificity of the assay for histidine decarboxylase activity was verified by incubating the samples with [β-^{3}H-(side chain carbon)]L-histidine (20 nCi) in addition to the ^{14}C-carboxyl-labeled L-histidine (20 nCi); the composition of the incubation mixture was otherwise identical to that used in the $^{14}CO_2$ release assay. After trapping the $^{14}CO_2$ in Hyamine, the reaction mixture was removed and assayed for [^{3}H]histamine by isotope dilution derivative analysis [17]. Endogenous histamine was assayed by an enzymatic isotopic assay [18] after dilution (1 to 10 parts by volume) of sonified cell suspensions with 0.1 M sodium phosphate buffer (pH 7.9).

Uptake of [β-^{3}H]L-histidine was measured by incubation of 10^5 mast cells (unless indicated otherwise) with 100 nCi (20 pmoles) [β-^{3}H]L-histidine in 100 μl of Hanks'–HEPES–albumin solution for 2.5 min (or as indicated) at 37° or 0°. Controls (blanks) contained unlabeled (10^{-2} M final concentration) histidine in addition to the labeled histidine. The cells were deposited by centrifuging the samples (8000 *g*) in a Microfuge (Beckman Instruments) through 250 μl fetal calf serum for 1 min. The supernatant fluid was removed by aspiration, and the tip of the centrifuge tube was cut off and placed in 5 ml Aquasol (New England Nuclear Corp.) for assay. All determinations were done in triplicate (three test and three blanks), and the difference in ^{3}H content of the test and blank pellets was designated as "uptake".

RESULTS

Histamine synthesis in intact and disrupted peritoneal mast cells. Separation of rat peritoneal cells into different fractions by elutriation yielded a single peak of cells containing histamine (Fig. 1). Mast cells contributed 80–85% of the cells in the fractions with maximum histamine content (mean ± S.E. histamine content, 16 ± 1.8 pg/mast cell, N = 5). The distribution of histidine decarboxylase activity paralleled that of histamine, although the rate of histamine formation by intact cells was much greater than that of soluble extracts of these cells (Fig. 1). This difference was of a similar magnitude whether

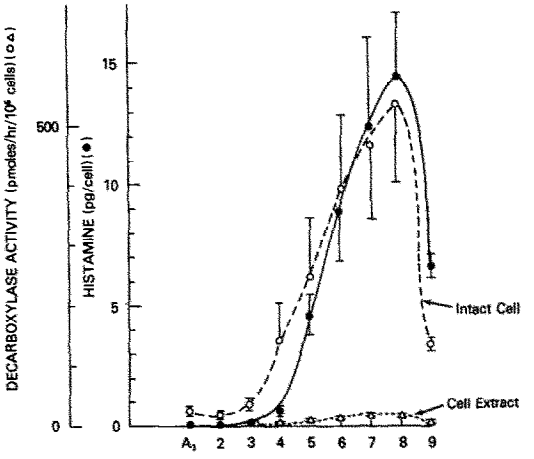

Fig. 1. Histamine content (●) and histidine decarboxylase activity (○, △) of the various fractions of peritoneal cells separated by elutriation. Histidine decarboxylase activity was measured by the $^{14}CO_2$ release in intact (○) and sonicated (△) cells. The points and bars depict mean and S.E. for three experiments. DOPA decarboxylase activity (not shown) was low (90–115% of blank values) in all fractions.

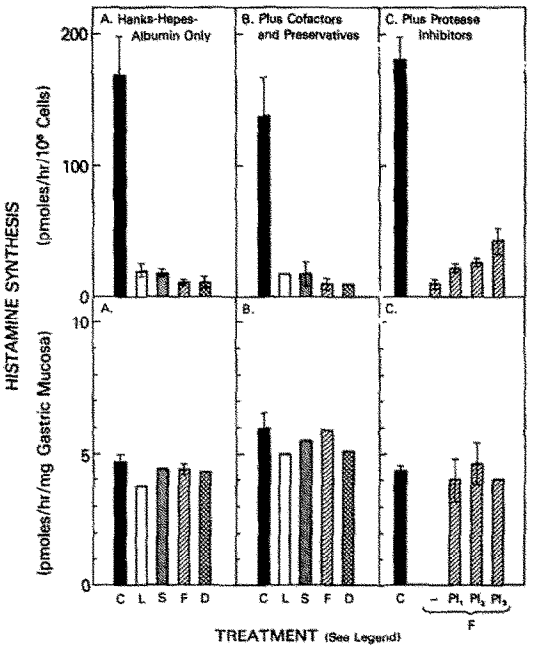

Fig. 2. Loss of histamine synthetic activity upon disruption of rat peritoneal mast cells by various procedures in the absence and presence of various inhibitors and preservatives. Columns (and bars) indicate mean (and S.E.) values for three to five experiments or mean value of two experiments. Mast cells were purified (86–96% mast cells) by centrifugation through Ficoll (upper panel A), and a soluble rat gastric histidine decarboxylase (lower panel B) was prepared as described under Materials and Methods. The cells (~5×10^6 cells) and the soluble gastric preparation (extract from 100 mg of gastric mucosa) were suspended in 1 ml of the medium indicated (see key below), and aliquots were subjected to various treatments before assay of histidine decarboxylase activity by the $^{14}CO_2$ release assay. For these assays, reagents were prepared with the different media and contained sufficient L-histidine and pyridoxal phosphate to give final concentrations of 2×10^{-5} M and 10^{-5} M respectively. Key: *Media:* (A) Hanks' medium with 50 mM HEPES, pH 7.2, and 0.1% (w/v) bovine serum albumin; (B) and (C) 0.1 M sodium phosphate buffer, pH 6.8, with 10^{-5} M pyridoxal phosphate, 2×10^{-4} M dithiothreitol, and polyethylene glycol 400 (1%, w/v). *Treatment:* (C) no treatment (■); (L) cells were first lysed (at 37° for 30 min) in water before dilution with medium (□); (S) sonification (▨); (F) freezing and thawing (on Dry Ice) three times (▨); (D) detergent [Triton X-100 (4%, w/v)] was added to suspensions of cells in the different media and the suspensions were incubated at 37° for 30 min (▩); (PI_1), phenylmethanesulfonyl fluoride (final concn, 100 μg/ml); (PI_2), pepstatin, antipain, leupeptin, and chymostatin (final concn, 20 μg/ml each); or (PI_3), soybean trypsin inhibitor (0.04%, w/v) were added prior to freezing and thawing of preparation three times.

histidine decarboxylase activity was measured by $^{14}CO_2$ release from ^{14}C-carboxyl-labeled histidine or by the formation of [^{3}H]histamine from side chain-labeled [β-^{3}H]histidine (Table 1). The decarboxylation of histidine was attributable to specific histidine decarboxylase in that the activity in cell extracts was inhibited by α-methylhistidine but not, except in high concentrations, by α-methylDOPA (see Table 2).

Loss of activity was evident when mast cells were disrupted by freezing and thawing, lysing with water, or detergent; when protease inhibitors, substrates (L-histidine and pyridoxal), or preservatives (polyethylene glycol 400 and Cleland's reagent) were present (Fig. 2); or in the presence or absence of sodium (data not shown). There was no such loss of activity with the soluble gastric enzyme preparation (Fig. 2) or when the gastric preparation was mixed with

Table 1. Decrease in histamine synthesis on disruption of rat peritoneal cells as measured by $^{14}CO_2$ release and histamine formation*

	N	% Label recovered as $^{14}CO_2$	% Label recovered as [β-^{3}H]Histamine
Peritoneal cells			
Intact	3	0.63 ± 0.03	0.64 ± 0.03
Sonicated	3	0.04 ± 0.01	0.03 ± 0.01
Peritoneal cells + Brocresine, (10^{-5} M)			
Intact	2	0.03	0.00

* Incubations (in duplicate) contained 125 μM histidine (^{14}C-carboxyl-labeled L-histidine plus [β-^{3}H]L-histidine), pyridoxal phosphate and mast cells (50,000 cells) as described in the text. The samples were incubated for 30 min. $^{14}CO_2$ was trapped in Hyamine hydroxide [16], and the final reaction mixture was assayed for [β-^{3}H]histamine by isotope dilution and formation of the benzene sulfonyl derivative of histamine [17]. Values are means ± S.E. and are corrected for assay blanks (medium only): 0.05% for $^{14}CO_2$ release, 0.01% for [β-^{3}H]histamine formation.

disrupted cell suspensions [34 ± 0.3 vs 42 ± 0.3 pmoles·hr^{-1}·(mg tissue)$^{-1}$].

Changes in kinetic parameters upon disruption. With intact cells, the rate of $^{14}CO_2$ release declined after 30 min, whereas with the extracts release was linear with time for up to 90 min. Assays with varying amounts of cells or cell extracts indicated that the rate of release was proportional to the amount of

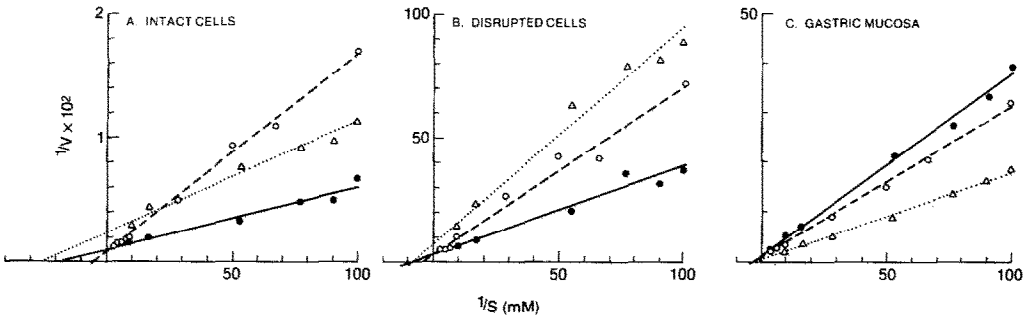

Fig. 3. Histidine concentration versus histamine synthetic activity of three preparations of intact (A) and disrupted (B) rat peritoneal mast cells and three gastric mucosal extracts (C). The different preparations are indicated by the symbols ●, ○ and △, and the data are presented in double-reciprocal plots. V equals pmoles of histidine decarboxylated as measured by $^{14}CO_2$ release per hr per 10^6 mast cells or mg gastric mucosa. Each point is the average value for three determinations and the lines of best fit, as determined by regression analysis, are shown. Analysis of the data by computer gave the following kinetic constants: for the intact mast cell, disrupted cell and gastric mucosal extracts, the apparent K_m values were, respectively, 90 ± 40, 160 ± 40 and 420 ± 80 μM and the values for V_{max} were respectively, 820 ± 210, 25 ± 6 and 120 ± 40 pmoles $^{14}CO_2$ released per hr per 10^6 mast cells or mg gastric mucosa. Because of the scatter of values in B—a reflection of the low activity in the disrupted mast cell preparations—the values for K_m are not regarded as reliable. The most striking difference is the large decrease in V_{max} upon cell disruption.

sample in amounts up to 0.5×10^5 cells/20 μl for intact cells or 4×10^5 cells/20 μl for cell extracts. The relationship of substrate concentration and histamine synthetic activity, as indicated by Lineweaver–Burk plots, showed that upon disruption of cells V_{max} decreased by more than 95% with no significant change in apparent K_m (Fig. 3). The apparent K_m for the soluble cell extract ($1.6 \pm 0.4 \times 10^{-4}$ M) was slightly lower than that of soluble extract of gastric mucosa ($K_m = 4.2 \pm 0.8 \times 10^{-4}$ M). Both the soluble mast cell and gastric enzyme, but not the histamine synthetic activity, of intact cells were partially dependent on pyridoxal phosphate, and optimum activity was observed with 10^{-5} M pyridoxal phosphate.

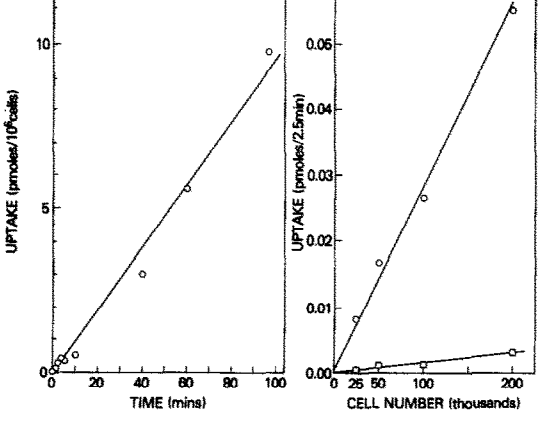

Fig. 4. Uptake of [^{3}H]histidine by purified peritoneal mast cells (○) and unfractionated peritoneal cells (□): (A) time course and (B) with increasing cell concentrations. Mast cells were purified by centrifugation through a Ficoll gradient and uptake was determined with 10^5 cells in (A) or varying numbers of cells (B) in a volume of 100 μl as described in Materials and Methods. Uptake was measured over a 2.5-min period in (B). Points show mean values of four determinations. Although incorporation of ^{3}H into protein was insignificant before 2.5 min, 59% of the ^{3}H was recovered in the protein precipitate by 100 min.

Histidine transport and effects of inhibition of transport. The rate of uptake of [^{3}H]histidine by purified mast cells was constant over the course of 100 min in one study (Fig. 4) but declined after 90 min (uptake by 90 min, 15.8 pmoles/10^6 cells) in a second study (data not shown). The rate of uptake was proportional to cell count (Fig. 4). Uptake was not observed with cells kept on ice and was blocked by glutamine, a substrate of the recently described histidine transport system ("N") [21] but not by methylaminoisobutyric acid, which is not a substrate for this system (Table 2).

The contribution of histidine uptake to the histamine synthetic activity of the intact cell was investigated by examining the effects of amino acids which might compete for the uptake of L-histidine. Of the various compounds tested, L-glutamine, L-tryptophan, 2-fluorohistidine, β-thienylalanine and, in high concentrations, α-methylDOPA inhibited decarboxylation as well as uptake of L-histidine in the intact cell but did not inhibit the histidine decarboxylase activity of cell extracts or the soluble gastric histidine decarboxylase preparation (Table 2). The decarboxylase inhibitors, α-aminohistidine (α-hydrazinohistidine) and Brocresine [19, 20], inhibited decarboxylase activity without interfering with L-histidine uptake, whereas α-methylhistidine inhibited both uptake and histamine synthesis in intact cells and histidine decarboxylation in the cell and gastric extracts (Table 2). Methylaminoisobutyric acid [21] did not inhibit uptake or decarboxylation of histidine.

Effects of disruption of cells from other sources. The marked reduction in histidine decarboxylation after sonification of cells was observed with mixed cell suspensions from the peritoneal and pleural cav-

Table 2. Effect of various compounds on L-histidine uptake and histidine decarboxylation in intact and sonified peritoneal mast cells and in gastric extract*

Compound	(M)	L-Histidine uptake: Intact cells	Histidine decarboxylase activity: Intact cells (% inhibition)	Disrupted cells (% inhibition)	Gastric extract (% inhibition)
Amino acids					
Glutamine	10^{-3}	77 ± 8	67 ± 3	5 (9, 0)	< 5
Glutamine	5×10^{-3}	91 ± 5	88 ± 7	9 (17, 0)	16
Tryptophan	10^{-4}	66 ± 12	45 ± 11	< 5 ± 0	< 5 ± 0
Tryptophan	10^{-3}	88 ± 5	75 ± 8	13 (11, 16)	21 ± 1
α-Methylaminoisobutyric acid	10^{-3}	10 ± 5	< 5	< 5 ± 0	
Histidine analogs					
2-Fluoro-L-histidine	5×10^{-4}	66 ± 3	52 (60, 44)	13 (17, 9)	14 ± 4
2-Fluoro-L-histidine	10^{-3}	90 ± 3	75 ± 10	28 (32, 23)	21 ± 4
β-Thienylalanine	10^{-4}	72 ± 10	64 (64, 64)	< 5 ± 0	7 ± 4
β-Thienylalanine	10^{-3}	76 ± 13	82 ± 3	6 (6, 6)	12 ± 4
Decarboxylase inhibitors					
α-Aminohistidine (α-Hydrazinohistidine)	10^{-3}	22 ± 4	98 (100, 96)	100	100
α-Methyl-L-histidine	10^{-4}	22 ± 7	39 (30, 48)	32	54 (54, 55)
α-Methyl-L-histidine	10^{-3}	67 ± 10	92 (94, 90)	81 ± 4	95 ± 1
α-Methyl-L-DOPA	10^{-4}	14 ± 9	30 (37, 23)	< 5 ± 0	< 5 ± 0
α-Methyl-L-DOPA	10^{-3}	86 ± 4	72 ± 6	14 ± 7	21 ± 1
Brocresine (NSD 1055)	10^{-5}	< 5 ± 0	100 ± 0	100 ± 0	100 ± 0

* Values are means ± S.E. where N is 4–7 or, in parentheses, individual values. All assays were done in triplicate. Histidine decarboxylase activity was measured by $^{14}CO_2$ release, and histidine uptake by the uptake of [β-^{3}H]histidine. These procedures were performed as described in Materials and Methods. Histidine decarboxylase activities in the absence of inhibitors were 178 ± 14 and 17 ± 4 pmoles per hr per 10^6 cells for intact and sonified mast cells and 5.0 ± 0.5 pmoles per hr per mg tissue for the rat gastric mucosal enzyme preparations (N = 9–12).

ities as well as with the purified mast cells. The loss of activity with partially purified preparation of ECL cells from rat gastric mucosa was less than that from the peritoneal and pleural cavities of the rat (Table 3). The reduction was the same whether mast cells were disrupted by freezing and thawing or sonification (data not shown).

DISCUSSION

Most tissues appear to produce the histamine contained in them [22]. Upon injection of isotopically labeled histidine, small amounts of labeled histamine appear in various tissues [3, 23–25] and are incorporated into endogenous stores [26]. Labeled his-

Table 3. Histamine synthesis in intact and disrupted cell preparations from different sources*

Source	Histamine synthesis† (pmoles/hr/10^6 cells): 2×10^{-5} M Histidine, Intact		2×10^{-5} M Histidine, Disrupted‡	2.5×10^{-4} M Histidine, Intact		2.5×10^{-4} M Histidine, Disrupted‡
(1) Pleural cavity (19% mast cells)				82	−93%	6 (1)
(2) Peritoneal cavity (11–19% mast cells)	8.3	−86%	1.2 (1)	34 ± 4	−82%	6 ± 1 (12)
(3) Mast cells (80–85%) from peritoneal cavity purified by elutriation				398 ± 25§	−95%	21 ± 4 (4)
(4) Mast cells (86–96%) from peritoneal cavity purified by Ficoll gradient	188 ± 26	−94%	12 ± 6 (3)	756§	−97%	23 (1)
(5) ECL cells (8–12%) from gastric mucosa purified by elutriation				93	−44%	52 (1)

* Each value is the mean or mean ± S.E. for the number (N) of preparations assayed. The percent decrease in activity is also noted.

† Histidine decarboxylase activity was assayed in triplicate by the $^{14}CO_2$ release assay in the presence of 2×10^{-5} or 2.5×10^{4} M L-histidine (see text).

‡ Cells were disrupted by sonification as described under Materials and Methods.

§ Equivalent to 51 and 97 pmoles·min^{-1}·(mg protein)$^{-1}$.

tamine and its metabolites are also excreted in the urine [27–30] along with the endogenous compounds [31–37]. Increases in the latter are associated with increased histamime production in the body in response to physiological and pathological changes [36, 37]. From these and studies with decarboxylase inhibitors *in vivo* [36, 38, 39], it has been concluded that histamine is formed continuously in most tissues at estimated turnover rates (half-life) of 50 days in the whole animal [26] to less than 1 hr in rat stomach [17] and brain [40]. Paradoxically, the activity of histidine decarboxylase in most tissues is extremely low. The standard assay procedures for this enzyme require incubation times of 3 hr or more [3]. Even in gastric mucosa, where histamine has an important role, histidine decarboxylase activity is, with the exception of rat [41], reported to be low [41, 42] or absent [43]. We suspect that these values do not reflect the actual rate of histamine synthesis *in vivo* and, as evident in the present study, significant loss of enzyme activity occurs upon homogenization of tissue.

The importance of studying synthesis in the intact cell as well as with isolated enzyme systems was recognized by Schayer in 1956, when he first reported the presence of a histidine decarboxylase activity in rat peritoneal cells. He noted that there was "invariably a drop (66–75%) in histamine-forming activity" when cells "were disrupted by repeated freezing and thawing or by sonification", and since a "fairly stable soluble histidine decarboxylase activity can be extracted from disrupted cells", he concluded loss of activity "might be due to the absence of processes afforded by the living cell which enhance histamine formation" [44]. Histidine transport may be a rate-determining process if it maintains high concentrations of substrate within the cell. Others have reported that with mixed suspensions of bone marrow cells histidine is taken up preferentially by basophils and converted to histamine [45]. Recently, Kilberg and associates have reported that histidine and glutamine are transported exclusively into rat hepatocytes by a Na^+-dependent system (System "N") which appears to be quite distinct from other amino acid transport systems (Systems "A", "L" and "ASC"). The uptake of both histidine and glutamine is by a single kinetic component, and both substrates compete for uptake [21]. The inhibition of histidine uptake by glutamine in our mast cell preparations suggests that a similar transport system exists in mast cells, and kinetic studies to be reported elsewhere indicate this to be the case. The present studies also indicate that two histidine analogs, 2-fluorohistidine and β-thienylalanine, like glutamine, compete with histidine transport. These three compounds, however, do not interfere with histidine decarboxylation in soluble extracts, and the determinants for attraction of substrate for histidine transport and histidine decarboxylase appear to be different. Although the data point to the possibility that interference in histidine uptake may be a mode of inhibiting histamine synthesis in intact tissue, a caveat is our uncertainty of the changes in size of the intracellular histidine pool during purification of the mast cell or the contribution of this pool to the overall kinetics of the assay system.

Histamine production by intact rat peritoneal mast cells was ten to thirty times greater than that of disrupted cells or soluble extracts of these cells. Destruction of enzyme rather than concentration of histidine within the cell seemed to us to be a more plausible explanation for this loss of activity and for the 90% decrease in values for V_{max}. Histidine levels in rat plasma (10^{-4} M) [11] and in our assay system (2.5×10^{-4} M, Table 3) would result in 50% or greater saturation of the enzyme, and no more than a 2-fold increase in the rate of histamine synthesis would be expected if there were further concentration of histidine within the cell. We were unable, however, to preserve enzyme activity by the treatments known to preserve histidine decarboxylase activity in partially purified enzyme preparations [5, 7] or by the use of inhibitors of proteolytic enzymes. These inhibitors, phenylmethylsulfonyl fluoride or a combination of leupeptin, antipain, chymostatin, and pepstatin, were reported by Yamada *et al.* [46] to prevent inactivation of histidine decarboxylase activity by pancreatic extracts, and they have used them to protect histidine decarboxylase in tissue extracts. These authors noted that, in the case of mast cells, the enzyme reaction was nonlinear after 20 min of incubation, and they suspected that the enzyme was still labile in the presence of these inhibitors. We, too, were unable to preserve full activity with the inhibitors, so we are uncertain as to the mechanism(s) responsible for loss of activity. The data do indicate that the intact mast cell has a high capacity for histamine synthesis and that histidine decarboxylase activity in both rat and dog (see Ref. 47) mast cells may be extremely labile, once cellular integrity is lost. Perhaps of significance, loss of activity was less upon disruption of the gastric ECL cells, which are of different origin than the mast cell, and a soluble histidine decarboxylase activity can be readily extracted from these cells [11]. This may indicate that the ECL cell lacks the proteolytic enzymes that have been identified in mast cells [48] or that its histidine decarboxylase activity differs from that in the mast cell.

ADDENDUM

Since submission of this manuscript, Bauza and Lagunoff [*Biochem. Pharmac.* **30**, 1271 (1981)] have reported that L-histidine is transported rapidly into rat peritoneal mast cells and converted to histamine. Their findings differ in that they assign the L system as the major system of histidine transport and report almost quantitative conversion of histidine to histamine: 86 and 78% within 1 min in the presence of 10^{-5} and 2.2×10^{-4} M L-histidine respectively (in incubations containing 10^6 cells in 150 μl). The percent conversions in our studies were 1.2% (in the presence of 2×10^{-5} M L-histidine) and 0.4% (2.5×10^{-4} M L-histidine) in 60 min in the presence of 5×10^4 cells in a volume of 40 μl.

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