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Amine Uptake into Intact Mast Cell Granules in Vitro†

Russell I. Ludowyke and David Lagunoff*

Department of Pathology, St. Louis University School of Medicine, St. Louis, Missouri 63104

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ABSTRACT: Histamine, the principal amine of rat peritoneal mast cells, is taken up into isolated granules with intact membranes. Uptake is pH- and concentration-dependent and is not stimulated by the addition of Mg^{2+}-ATP. The saturable uptake has a K_m of 91.1 μM and a V_{max} of 95.4 pmol (mg of protein)$^{-1}$ min^{-1}. Uptake is abolished by 5 mM ammonium ion. 5-HT, the other endogenous amine of the granules, and dopamine and tyramine, which do not occur naturally in rat mast cells, each competitively inhibits [^{3}H]-histamine uptake with K_i's close to 1 μM. Reserpine, a putative amine carrier blocker, inhibits uptake at nanomolar concentrations. At high concentrations, uptake of [^{3}H]-5-HT is nonsaturable; at low concentrations, a saturable component is observed with a K_m of 1.6 μM. Uptake of [^{3}H]-5-HT is not enhanced by Mg^{2+}-ATP. It is pH-dependent but with a lower apparent pK_a than that of histamine. [^{3}H]-5-HT uptake can be completely inhibited by ammonium ions. Amine inhibition of [^{3}H]-5-HT uptake gives nonlinear Dixon plots, and high concentrations of the competing amines or reserpine cannot completely block uptake. We propose a model consistent with these results in which amine uptake occurs by several distinct saturable transport systems. According to the model, histamine is transported by a single system, which also transports 5-HT and dopamine. 5-HT and dopamine are transported by one or more other systems.

Connective tissue mast cells of the rat contain histamine and 5-HT. These amines, located in the cell's specific granules, are secreted exocytotically and coordinately in response to a variety of agents (Lagunoff et al., 1983).

The mechanism by which amines enter secretory granules, though unexplored for mast cell granules, has been previously studied in chromaffin granules and platelet-dense granules. Current evidence suggests that a Mg-dependent ATPase, present in the chromaffin and the dense granule membrane, creates an electrochemical gradient comprised of both a pH

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gradient and a transmembrane electrical potential (Johnson & Scarpa, 1979; Carty et al., 1981). A reserpine-sensitive carrier with a broad specificity for amines (Slotkin et al., 1975) is responsible for the electrochemically driven passage of the amines through the granule membrane.

Rat mast cell granules differ from chromaffin granules and platelet-dense granules in both the high ratio of histamine to other amines and the large absolute quantity of histamine stored. The study of amine transport by mast cell granules was prompted by these considerations. The availability of a method for the preparation of mast cell granules with intact membranes (Kruger et al., 1980) made the study feasible.

Materials and Methods

Materials were obtained from the indicated sources as follows: bovine albumin (Path-O-Cyte 4) from Miles Laboratories Inc. (Elkhart, IN); Percoll from Pharmacia Fine Chemicals (Piscataway, NJ); tyramine hydrochloride from Calbiochem (La Jolla, CA); [^{3}H]-histamine (5.8 Ci/mmol) from New England Nuclear (Boston, MA); [^{3}H]-5-hydroxytryptamine creatinine sulfate (12.3 Ci/mmol) from Amersham (Arlington Heights, IL); [^{3}H]dopamine (53 Ci/mmol) from Research Products Int. (Mt. Prospect, IL); dopamine hydrochloride, histamine dehydrochloride, 5-hydroxytryptamine creatinine sulfate, and ATP (sodium salt, grade 1, crystalline) from Sigma Chemical Co. (St. Louis, MO).

Mast cells were obtained from male Sprague-Dawley rats (400–500 g) (Sasco, Omaha, NE) by peritoneal lavage and collection as previously described (Lagunoff, 1975). A population of 95–98% pure mast cells was achieved following centrifugation at 225g, for 15 min through a Percoll gradient (7 mL of Percoll with 1.0 mL of 10 × concentrated salts solution containing 1.54 M NaCl, 27 mM KCl, and 9 mM $CaCl_2$, plus 0.5% albumin, adjusted to pH 7.2 with 0.1 M KH_2PO_4 and final volume made up to 10.0 mL). The purified mast cells were washed twice in balanced salts solution containing albumin (BSSA; diluted salts as above were adjusted to pH 7.2 with 0.1 M phosphate buffer, and 0.5% albumin was added) and resuspended in 2.0 mL of BSSA for sonication [(2–3) × 10^6 mast cells/mL]. Sonication was effected in 16-mm test tubes in a bath sonicator (Sonogen, Branson Instruments Inc., Stamford, CO), by two 15-s bursts with a 30-s cooling period between. This method resulted in few remaining intact mast cells and a 50–70% recovery of histamine in the intact granules. The suspension was centrifuged at 150g for 5 min, and the supernatant stored on ice, and the pellet washed twice in 2.0 mL of BSSA. Three- or four-milliliter aliquots of combined granule suspensions were carefully layered on a second solution of Percoll [4.5 mL diluted with 0.5 mL of concentrated salts as above in 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), with 0.5% BSA, adjusted to pH 7.2–7.4] and centrifuged in a Sorvall SS-34 angle rotor at 27000g for 20 min. Intact, membrane-bound granules were present in a distinct band (C band) near the bottom of the 12-mL tube, whereas granules that lacked membranes remained in a band (A band) near the interface. The C band granules were carefully removed, diluted with 5 mL of medium, collected by centrifugation at 2100g in a swing-out rotor in an IEC-PRJ centrifuge for 15 min, and then used promptly for uptake experiments.

Amine uptake was measured in a final volume of 120 μL of sucrose/Hepes (S/H; 275/50 mM) in 10-mL polypropylene tubes incubated at 37 °C for 10 min, unless indicated otherwise. Uptake was terminated with the addition of 5 mL of ice-cold buffer, and the granules were collected on a 0.2 μM pore cellulose membrane filter by vacuum filtration. Filters were washed twice with 3.0 mL of ice-cold S/H buffer, dried, and counted in 10 mL of scintillation fluid in a Beckman LS-250 liquid scintillation spectrometer. In all experiments, parallel incubations of granules were performed on ice to correct for nonspecific binding of amine to granules and to the filters. Total granule histamine was assayed spectrofluorometrically via the OPT reaction as described previously (Lagunoff, 1975), and granule protein was measured by a modification of the Lowry method. Ruthenium red binding to granule matrices was used to measure membrane integrity (Kruger et al., 1980).

Table I: Stability of Granules[a]

medium	incubation time (min)	% spontaneous histamine release temperature 4 °C	22 °C	37 °C
sucrose/Hepes (S/H)	15	8 (1)	10 (1)	8 ± 2 (5)
	30	4 (1)	7 (2)	10 ± 1 (11)
	60	9 (2)	14 (2)	11 ± 1 (13)
BSSA	30	ND	ND	35 ± 6 (3)
	60	22 (2)	25 (2)	53 ± 5 (4)

[a] Isolated intact mast cell granules were suspended in media (450 μL, pH 7.2) for indicated times and temperatures. The sucrose/Hepes results at 37 °C are at concentrations of 275/50 mM; the others are at 300/10 mM. Final spontaneous histamine release was determined by the amount of histamine in the supernatant following centrifugation at 2100g for 12 min, as compared with the total present. Figures in parentheses indicate the number of experiments; where there are more than two, means ± SE are given (ND = not done). BSSA = balanced salts solution containing albumin.

Results

In preliminary experiments, intact granules were resuspended and incubated in BSSA (pH 7.2) for various times. Under these conditions, the granules were unstable, losing over 50% of their endogenous histamine during a 60-min incubation (Table I). Loss of membrane integrity was demonstrated by a concurrent increase in ruthenium red binding to the granule matrix. When intact granules were incubated in solutions of 275 mM sucrose buffered with Hepes adjusted to pH 7.2 with KOH, less than 15% of their histamine was released over 60-min incubation at various temperatures (Table I) and less than 10% over the usual incubation period of 10 min. Ruthenium red binding also remained low, consistent with the retention of histamine. Sucrose/Hepes (S/H), 275/50 mM, solution was therefore used routinely in uptake experiments.

When intact granules were incubated with [^{3}H]histamine (1.25 μM; 0.9 μCi) in S/H, pH 8.0 at 37 °C, uptake was linear over the first 10 min; subsequently, the rate decreased progressively (Figure 1). There was little uptake of [^{3}H]histamine when granules were incubated at 0 °C. The effect of ambient pH on [^{3}H]histamine uptake was determined by incubation of granules in S/H of different pH (Figure 2).

The concentration dependence of [^{3}H]histamine uptake was determined by adding increasing concentrations of unlabeled histamine. Uptake was saturable (Figure 3), and a least-squares analysis of the linear Lineweaver–Burk plot yields a K_m of 91.1 μM and a V_{max} of 95.4 pmol (mg of protein)$^{-1}$ min^{-1}.

The addition of Mg-ATP to isolated chromaffin granules greatly stimulates catecholamine accumulation (Johnson & Scarpa, 1979). It also stimulates 5-HT uptake into intact platelet-dense granules or granule ghosts by 2–3-fold (Carty et al., 1981; Wilkins & Salganicoff, 1981) and histamine uptake into platelet-dense granules by 3-fold (Fukami et al., 1984). In our hands, Mg-ATP did not stimulate the [^{3}H]-histamine uptake into mast cell granules over incubation periods of 10–60 min (Table II). Furthermore, neither the

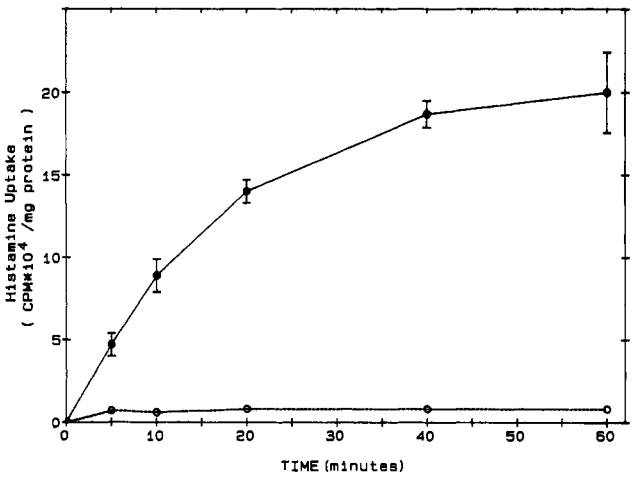

FIGURE 1: Rate of histamine uptake. Intact mast cell granules were suspended in S/H (275/50 mM; pH 8.0) containing [^{3}H]histamine (1.25 μM, 0.9 μCi). The granules were incubated at 0 or 37 °C for various times from 5 to 60 min. Values represent 10^4 cpm/mg of protein and are means ± SE of duplicate measurements in at least three experiments. The lower set of values are those obtained at 0 °C.

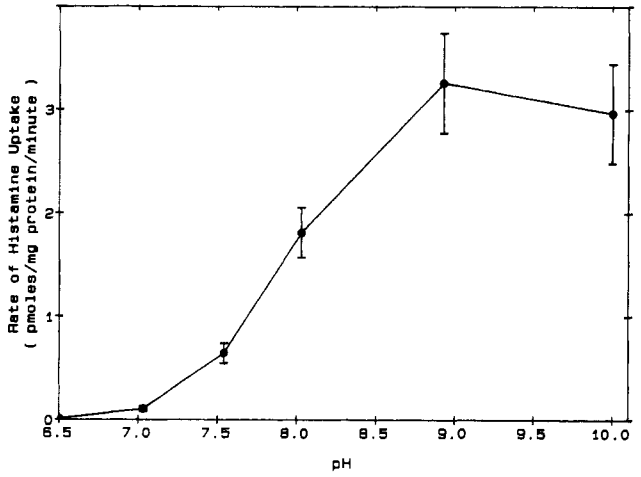

FIGURE 2: pH dependence of histamine uptake. Uptake of [^{3}H]-histamine (1.25 μM) by mast cell granules in S/H at pHs varying from 6.5 to 10. K^+ concentration was maintained at 65 mM by reducing KCl as KOH was increased. Values represent rate of uptake of [^{3}H]histamine [pmol (mg of protein)$^{-1}$ min^{-1}] and are means ± SE of duplicate measurements from at least three experiments. Control values for nonspecific binding have been subtracted from all results.

Table II: Effect of ATP on Amine Uptake[a]

amine	ATP/$MgSO_4$ (5 mM)	incubation time (min)	mean uptake ± Se (cpm × 10^4/ mg of protein)	n
histamine	−	10	6.7 ± 0.3	5
	+	10	4.6 ± 0.4	5
	−	30	12.7 ± 1.1	5
	+	30	11.3 ± 1.3	5
	−	60	17.4 ± 1.5	4
	+	60	13.1 ± 2.9	4
5-HT	−	10	55.1 ± 5.7	5
	+	10	46.6 ± 5.9	5
	−	30	73.1 ± 3.7	5
	+	30	69.1 ± 4.1	5
	−	60	95.7 ± 7.4	4
	+	60	86.5 ± 9.8	4

[a] Intact mast cell granules were suspended in S/H and ATP/$MgSO_4$ added to a final concentration of 5 mM and pH of 8.0. Granules were incubated at 37 °C for 10, 30, or 60 min before uptake was terminated as described under Materials and Methods. All values are means of duplicates, and figures in parentheses represent the number of experiments (histamine, 1.25 μM; 5-HT, 0.34 μM).

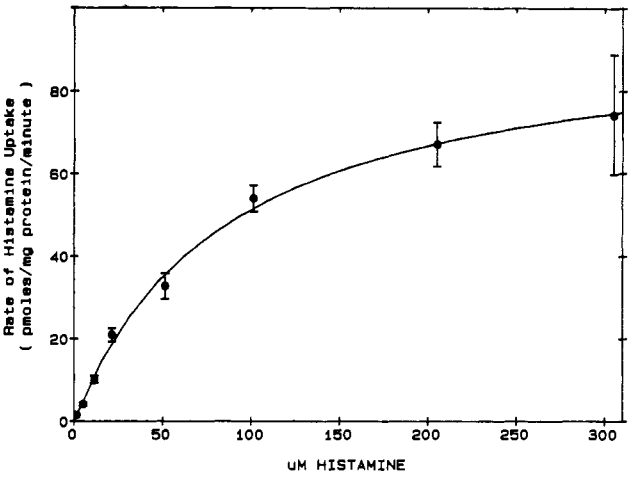

FIGURE 3: Concentration dependence of histamine uptake. Uptake of histamine was determined in S/H, pH 8.0. Values represent the rate of uptake of histamine [pmol (mg of protein)$^{-1}$ min^{-1}] and are means ± SE of duplicate measurements in at least three experiments. Concentrations given are total free base. The continuous line was calculated from the parameters determined from the Lineweaver–Burk plot fitted by the method of linear least squares. K_m = 91.1 μM and V_{max} = 95.4 pmol (mg of protein)$^{-1}$ min^{-1}.

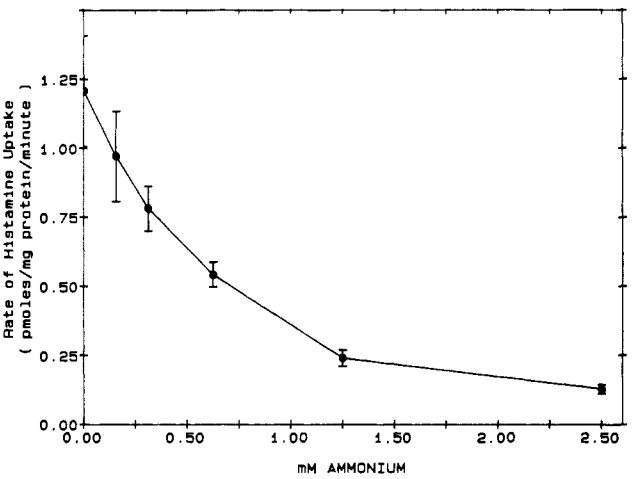

FIGURE 4: Ammonium ion inhibition of histamine uptake. Granules were incubated at 22 °C for 15 min in S/H, pH 8.0, containing varying concentrations of NH_4Cl. Values represent the subsequent [^{3}H]-histamine (1.25 μM) uptake [pmol (mg of protein)$^{-1}$ min^{-1}] at 37 °C and are means ± SE of duplicate measurements (minus control values) from at least three experiments.

addition of 5 mM Ca^{2+} nor the addition of Cl^- in the presence of Mg-ATP had any stimulatory effect on amine uptake.

Ammonium, an ion capable of collapsing transmembrane proton gradients by permeating the membrane in an uncharged form and alkalinizing the interior by taking up a proton (Johnson et al., 1981), was tested for its effect on [^{3}H]histamine uptake. Granules were preincubated in S/H to which NH_4Cl was added 15 min before the addition of the labeled amine. This strategy resulted in a NH_4^+ concentration-dependent reduction of the uptake of [^{3}H]histamine (Figure 4). At ammonium concentrations above 5 mM, a concentration-dependent increase in ruthenium red binding was observed, indicating loss of granule membrane integrity.

The effect on the uptake of [^{3}H]histamine of increasing concentrations of 5-HT, the other major amine in mast cells, and two other related amines, dopamine and tyramine, was examined. All three amines inhibited the uptake of [^{3}H]-histamine in the micromolar range, and in each case, inhibition was consistent with a simple competitive mode of action. K_i's determined from linear least-squares analysis of Dixon plots

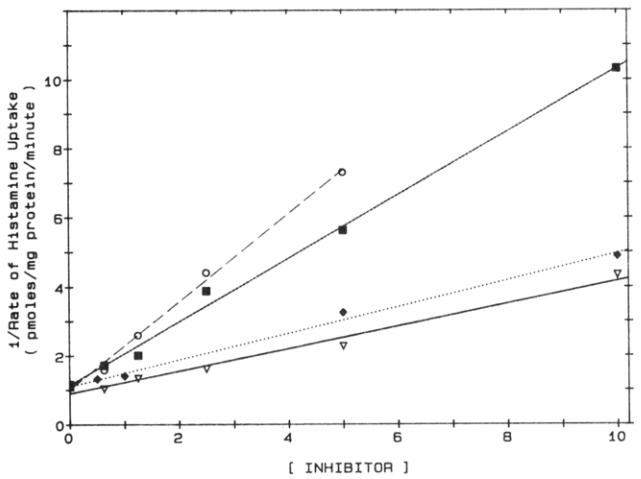

FIGURE 5: Inhibition of histamine uptake by amines and reserpine. Uptake of [^{3}H]histamine (1.25 μM) was determined in S/H, pH 8.0. The amines are readily soluble in the S/H. Reserpine was dissolved in 10 μL of glacial acetic acid and diluted in absolute ethanol. Final ethanol concentration was 0.83%. Uptake was compared with controls incubated with the same concentration of ethanol. Values represent the reciprocal of the rate of uptake of histamine (1/[pmol (mg of protein)$^{-1}$ min^{-1}]) and are means of duplicate measurements (minus control values) in at least three experiments. All amine concentrations are in micromolar values, whereas reserpine concentrations are in nanomolar values. Tyramine (○); 5-HT (■); reserpine (◆); dopamine (▽). Continuous lines for the Dixon plots were determined by the method of linear least squares.

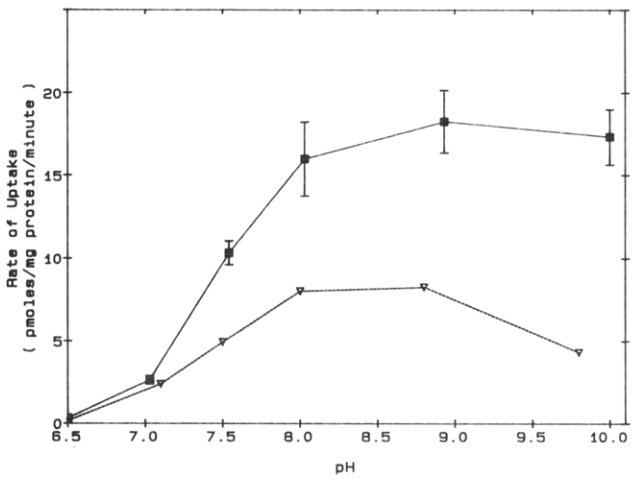

FIGURE 6: pH dependence of amine uptake. Uptake of [^{3}H]amine by mast cell granules in S/H at pHs varying from 6.5 to 10. K^+ concentration was maintained at 65 mM by reducing KCl as KOH was increased. Values represent rate of uptake of amine [pmol (mg of protein)$^{-1}$ min^{-1}] and are means ± SE of duplicate measurements from at least three experiments for 5-HT and means of two experiments for dopamine. Control values have been subtracted from all results. 5-HT (0.34 μM) (■); dopamine (0.04 μM) (▽).

(Figure 5) were 1.2, 0.8, and 2.7 μM for 5-HT, tyramine, and dopamine, respectively.

Reserpine, an agent considered to inhibit the uptake of amines into chromaffin granules by binding to the amine carrier (Scherman & Henry, 1980; Phillips, 1978), was tested for its effects on [^{3}H]histamine uptake by mast cell granules and found to competitively inhibit uptake in the nanomolar range with a K_i of 2.8 nM as determined from the Dixon plot (Figure 5). The competitive inhibition of [^{3}H]histamine uptake by the related amines and reserpine suggested that, as in other granule systems, the amines all bound to a single putative amine carrier. To confirm this, the uptake of [^{3}H]-5-HT and [^{3}H]dopamine, which is present in the mast cells of ruminants, was investigated.

The uptake of [^{3}H]-5-HT (0.34 μM; 0.5 μCi) was essentially similar to that of histamine, with linear uptake over the first 10 min and little uptake at 0 °C. Uptake of [^{3}H]dopamine (0.04 μM; 0.25 μCi) was only linear over the first 5 min, and all uptake experiments with [^{3}H]dopamine were therefore terminated after 5 min. Furthermore, uptake of [^{3}H]dopamine at 0 °C was substantial, amounting to 20% of that at 37 °C.

The pH profiles for the uptake of [^{3}H]-5-HT and [^{3}H]-dopamine were similar to that of [^{3}H]histamine, but they both had lower apparent pK_a's (Figure 6). The concentration dependence of [^{3}H]-5-HT uptake as determined by adding increasing concentrations of unlabeled 5-HT demonstrated two components of the curve (Figure 7). The first component, predominant at low amine concentration, was saturable with a K_m of 1.6 μM and a V_{max} of 34.2 pmol (mg of protein^{-1} min^{-1} as determined by a Lineweaver–Burk plot. The second component, at high amine concentration, was nonsaturable and increased linearly with 5-HT concentration such that the rate of uptake per minute equaled 0.42 × 5-HT concentration. At the standard concentration of [^{3}H]-5-HT used (0.34 μM), the nonsaturable component contributes only 2% of the total uptake.

The concentration dependence of [^{3}H]dopamine uptake, like that of histamine, exhibited one saturable component, and a least-squares analysis of the Lineweaver–Burk plot gave a K_m of 1.6 μM and a V_{max} of 258 pmol (mg of protein^{-1} min^{-1} (Figure 8). Analysis of the kinetics of the uptake of [^{3}H]-dopamine however is confounded by the fact that the uptake at 0 °C also exhibits one saturable component, with a K_m of 1.5 μM and a V_{max} of 52.4 pmol (mg of protein^{-1} min^{-1}; the uptake at 0 °C is also pH dependent.

As was observed for the uptake of [^{3}H]histamine, the addition of 5 mM Mg-ATP did not stimulate the uptake of [^{3}H]-5-HT over incubation periods up to an hour (Table II) and likewise did not stimulate [^{3}H]dopamine uptake (data not shown). The dependence upon a pH gradient for [^{3}H]-5-HT uptake was demonstrated by the inhibition of uptake following preincubation of the granules with NH_4Cl. Dopamine uptake was relatively insensitive to the NH_4^+ ion effect so that at 2.5 mM NH_4Cl, which inhibited uptake of [^{3}H]histamine and [^{3}H]-5-HT by 90%, [^{3}H]dopamine uptake was inhibited only 34%.

The inhibitory effects of reserpine and the amines histamine, dopamine, and tyramine on [^{3}H]-5-HT uptake were examined. It was determined that they all inhibited [^{3}H]-5-HT uptake, but in each case the Dixon plots were curved and the uptake could not be driven to zero by high concentrations of inhibitor. This different form of inhibition suggested that more than one uptake site might exist for 5-HT. To further elucidate this point, combinations of the different inhibitors in concentrations many fold greater than their respective K_i's for histamine uptake were examined for their inhibitory effect on the uptake of [^{3}H]-5-HT and [^{3}H]dopamine. Since incomplete inhibition was evident at low concentrations of [^{3}H]-5-HT, this effect was not dependent on the nonsaturable component of 5-HT uptake.

The results in Table III show that histamine, reserpine, or a combination of the two only inhibited [^{3}H]-5-HT uptake by 60%. Dopamine alone achieved 80% inhibition. the uptake of [^{3}H]dopamine however was inhibited only 22% by any of the other amines including 5-HT and reserpine, either acting alone or in concert.

Discussion

The effect of moderately hyperosmolar (360 mosm) solutions of sucrose to prevent osmotic lysis of mast cell granules

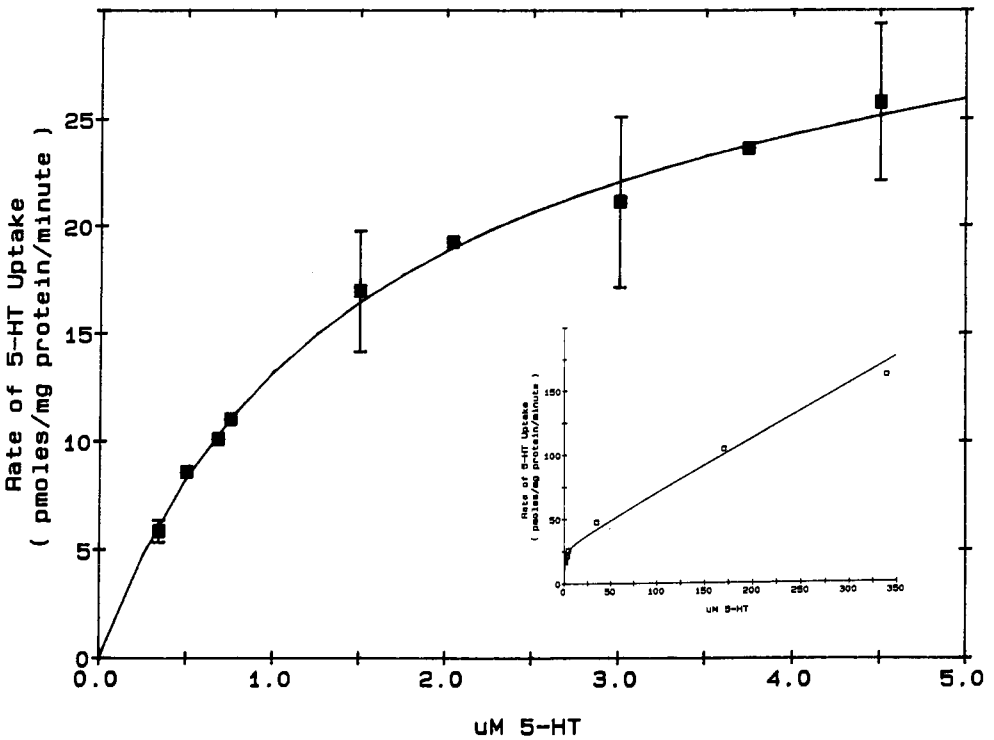

FIGURE 7: Concentration dependence of 5-HT uptake. Uptake of 5-HT was determined in S/H, pH 8.0. Values represent the rate of uptake of 5-HT [pmol (mg of protein)$^{-1}$ min^{-1}] and are means of duplicate measurements (minus control values) in at least two experiments. Standard errors are given where three or more experiments were considered. The inset presents the data over the entire range of concentrations tested. The continuous lines are calculated for the sum of saturable and nonsaturable components according to the equation rate $V_{max}[S]/(K_m + [S]) + K_d[S]$, where K_m = 1.6, V_{max} = 34.2, K_d = 0.42, and [S] = substrate concentration.

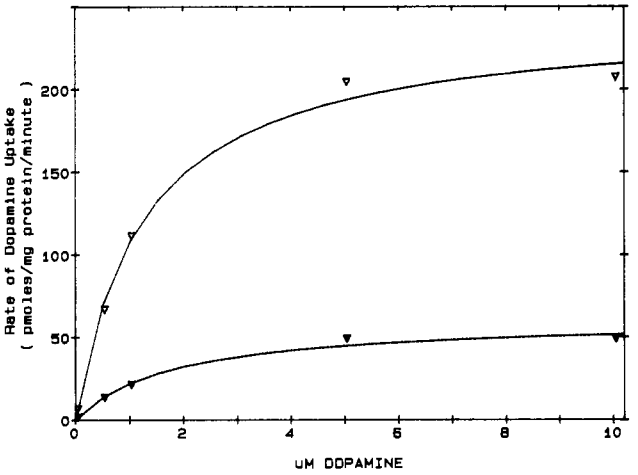

FIGURE 8: Concentration dependence of dopamine uptake. Uptake of dopamine was determined in S/H, pH 8.0. Values represent the rate of uptake of dopamine [pmol (mg of protein)$^{-1}$ min^{-1}] and are means of duplicate measurements (minus filter controls) in two experiments. The top curve (▽) represents uptake at 37 °C and the bottom (▼) uptake at 0 °C. The lines were calculated with the parameters determined from the Lineweaver–Burk plot fitted by the method of linear least squares. K_m = 1.6 μM, V_{max} = 258 pmol (mg of protein)$^{-1}$ min^{-1} at 37 °C and 1.5 and 52.4, respectively, at 0 °C.

Table III: Inhibition of Amine Uptake[a]

competing amine	K_i of [^{3}H]histamine	% inhibition of uptake [^{3}H]-5-HT (n)	% inhibition of uptake [^{3}H]dopamine (n)
histamine (1 mM)	(K_m = 91.1 μM)	56 ± 4 (9)	21 ± 2 (3)
5-HT (50 μM)	1.2 μM	(K_m = 1.6 μM)	23 ± 2 (3)
dopamine (50 μM)	2.7 μM	81 ± 4 (6)	(K_m = 1.6 μM)
reserpine (100 nM)	2.8 nM	63 ± 4 (6)	22 ± 3 (3)
reserpine + histamine		65 ± 6 (3)	23 ± 2 (3)
reserpine + dopamine		77 ± 2 (3)	
histamine + dopamine		76 (2)	
reserpine + 5-HT			21 ± 2 (3)
histamine + 5-HT			23 ± 2 (3)
reserpine + histamine + dopamine		78 (2)	
reserpine + histamine + 5-HT			23 ± 1 (3)

[a] K_m values were determined from linear least-squares analysis of Lineweaver–Burk plots. K_i values for inhibition of histamine uptake were similarly determined from linear Dixon plots. Values represent percent inhibition of [^{3}H]-5-HT (0.34 μM) or dopamine (0.04 μM) uptake by other amines and reserpine, along or in combination, and are means of duplicates (minus control values). Where three or more experiments were considered (n), standard errors are given. Inhibitory concentrations used: histamine (1 mM); 5-HT (50 μM); dopamine (50 μM); reserpine (100 nM).

has been observed previously for platelet-dense granules (Wilkins et al., 1978) and chromaffin granules (Holz, 1980). A number of other parallels are evident between amine uptake by intact mast cell granules and other storage granule systems. A short period of linear uptake with the rate progressively decreasing thereafter is common for 5-HT uptake in platelet granules (Wilkins et al., 1978), insulin granules (Hutton et al., 1983), brain synaptic vesicles (Maron et al., 1979), and chromaffin granule membranes (Phillips, 1978) and for [^{3}H]histamine uptake into platelet-dense granules (Fukami et al., 1984) although the duration of the first component of uptake varies. Leakage of amines from mast cell granules did not account for the fall off in rate.

The lack of the reqiurement for Mg-ATP for amine uptake into mast cell granules is somewhat surprising as it markedly

stimulates catecholamine accumulation into isolated chromaffin granules (Johnson & Scarpa, 1979) and stimulates by 2–3-fold amine uptake into platelet-dense granules or granule ghosts (Carty et al., 1981; Wilkins & Salganicoff, 1981; Fukami et al., 1984).

It has been well established that a membrane-bound Mg-ATPase pumps protons into chromaffin granules (Njus et al., 1981) and platelet-dense granules (Given & Longenecker, 1985). The pump has been characterized (Johnson et al., 1982) and recently isolated from chromaffin granule membranes (Percy et al., 1985). In both granule systems, maximal rates of amine uptake were observed in the presence of both a transmembrane proton gradient (ΔpH) and a potential gradient ($\Delta\Psi$), although either could independently drive amine accumulation (Johnson & Scarpa, 1979; Apps et al., 1980; Carty et al., 1981). Recently, Kanner and Bendahan (1985) described a Mg-ATP-dependent uptake system for 5-HT in membrane vesicles from rat basophilic leukemia cells. In mast cells there is some limited evidence for a low-affinity Mg- or Ca-dependent ATPase in intact granule membranes (Chakravarty & Nielsen, 1980; Amende & Donlon, 1985). The explanation for our results most likely lies in the observation that mast cell granules maintain a gradient of at least 1 pH unit across the granule membrane in the absence of ATP (Lagunoff & Rickard, 1983). The highly acidic heparin in the mast cell granule matrix would be expected to maintain a significant proton gradient on the basis of a Donnan effect, as long as the granule membrane is poorly permeable to K^+. Furthermore, the imidazole ring of histamine is capable of acting as a buffer in the vicinity of its pK_a of 6.0–6.4 and maintaining an acid environment once established within the granules, in the absence of an active ATP-driven H^+ pump. The dependence of amine uptake upon the pH gradient was strongly suggested by the sensivity of uptake to NH_4^+. Further investigations of the driving force for amine transport by mast cell granules will require actual measurements of the pH gradient and the electrical potential across the granule membrane.

The K_m observed here for [^{3}H]-5-HT uptake into mast cell granules (1.6 μM), is of similar magnitude to that in chromaffin granules (4.3 μM), brain synaptic vesicles (1.0 μM) (Kanner et al., 1979; Maron et al, 1979), platelet-dense granules (3.3 μM), granule ghosts (1.2 μM) (Wilkins et al., 1978; Fishkes & Rudnick, 1982), and membrane vesicles of rat basophilic leukemia cells (2.6 μM) (Kanner & Bendahan, 1985). The V_{max} we have determined for 5-HT uptake into mast cell granules [34 pmol (mg of protein^{-1}) min^{-1}] is lower than those observed in these other systems; however, the basis for comparison in terms of protein content of granules is likely to be inappropriate because of large differences in protein content of the granule matrix unrelated to the quantity of membrane protein per organelle.

Histamine uptake into mast cell granules appears to have only one kinetic component, with a considerably higher K_m (91.1 μM) than that observed for uptake of either 5-HT or dopamine. The low affinity for histamine measured in terms of uptake or inhibition of the uptake of other amines compared with that for 5-HT and dopamine has been observed in chromaffin granules (Kirshner, 1962; DaPrada et al., 1975) and platelet-dense granules (DaPrada & Pletscher, 1969; Fukami et al., 1984). The histamine content of rabbit platelet-dense granules is about one-third that of 5-HT (DaPrada & Pletscher, 1969), but in porcine platelet-dense granules, histamine content is higher than that of 5-HT (Urgurbil et al., 1984). In the porcine granules, the rate of histamine uptake is 80–100-fold less than that of 5-HT (Fukami et al., 1984).

In rat peritoneal mast cells where the histamine content is about 80 times that of 5-HT (Wingren et al., 1983), it has been demonstrated that although histamine is taken up by the cells slowly, histidine crosses the plasma membrane readily and is very rapidly converted to histamine and, subsequently, quickly taken up by the granules (Bauza & Lagunoff, 1981), as opposed to platelets where the adsorbed histamine is not immediately sequestered into the dense granules (Fukami et al., 1984). Mast cells are very long-lived cells, and under normal in vivo conditions, the turnover of histamine, 5-HT, or heparin is slow (23, 25, and 35 days, respectively) (Wingren et al., 1983). Following the exocytotic release of its contents, it takes about 21 days for the peritoneal mast cell to recover its store of histamine (Kruger & Lagunoff, 1981).

That histamine was transported into the granule by a relatively nonspecific amine carrier was demonstrated by complete competitive inhibition by 5-HT, dopamine, and tyramine. The K_i's in the vincinity of 1 μM for the amines correspond to those observed with platelet-dense granules (Wilkins et al., 1978). The lack of total inhibition of [^{3}H]-5-HT and [^{3}H]-dopamine uptake by concentrations of competitive amines and reserpine far in excess of their K_i's for [^{3}H]histamine uptake suggests the presence of other amine carriers (Table III). As histamine, reserpine, or a combination of the two could only inhibit 60% of the total [^{3}H]-5-HT uptake, and no additive effect was seen; the most reasonable explanation is that reserpine reacts exclusively with the histamine carrier. An addition 20% inhibition of 5-HT uptake by dopamine suggests that at least two 5-HT uptake sites in addition to the histamine carrier exist, one of which is dopamine sensitive and one which is insensitive to any of the amines tested. However, 5-HT does not inhibit the uptake of [^{3}H]dopamine to any greater extent than histamine or reserpine. This suggests that whereas dopamine blocks one of the 5-HT carriers, it is itself not taken up at that site. Thus, 80% of the dopamine uptake appears to occur at a site or sites or by a mechanism distinct from the histamine carrier. The possibility of oxidation of dopamine in alkaline media and the incomplete inhibition by concentrations of NH_4^+ sufficient to block both histamine and 5-HT uptake complicate the analysis of dopamine uptake. Preliminary investigations of the anomalous uptake of [^{3}H]dopamine occurring at 0 °C revealed that over a 20-min period uptake increased steadily with time. Uptake was also pH dependent at 0 °C. Whereas 50 μM 5-HT inhibited [3H]dopamine uptake at 37 °C by 23%, no inhibition was observed at 0 °C. The K_m's of uptake at 37 and 0 °C are the same, with the V_{max} at 0 °C being $^1/_5$ that at 37 °C. One possible explanation of these results is that the specific dopamine carrier is far more functional at 0 °C than the carriers responsible for histamine and 5-HT uptake. Since carrier function is a priori unlikely at 0 °C, the possibility that the apparently saturable uptake is not carrier mediated must be considered. The failure of reserpine to block the major component of dopamine uptake is also inconsistent with carrier-mediated uptake of this amine. High-performance liquid chromatographic analysis of rat mast cell lysate with electrochemical detection revealed expected amounts of 5-HT but no dopamine (unpublished).

An unsuccessful attempt was made to discriminate between the several putative carrier mediated 5-HT uptake systems on the basis of their kinetic parameters. With the data available, no significant differences in goodness of fit were obtained when the best fit curves, assuming two or three systems, were compared to the curve calculated for a single system with a K_m

of 1.4 μM and a V_{max} of 30.4 pmol (mg of protein)$^{-1}$ min^{-1}. This result is presumably dependent on the closeness of the values for K_m's and V_{max}'s of the several proposed sites relative to the precision of the data.

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Registry No. 5-HT, 50-67-9; histamine, 51-45-6; dopamine, 51-61-6; tyramine, 51-67-2.

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