Quantal Corelease of Histamine and 5-Hydroxytryptamine from Mast Cells and the Effects of Prior Incubation†

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ABSTRACT: Corelease of histamine and 5-hydroxytryptamine from individual mast cells has been measured with fast-scan cyclic voltammetry using a carbon-fiber electrode placed next to a single cell. Release events, induced by exposure of the cells to the calcium ionophore, A23187, were resolved at the level of individual exocytotic events. Changes in the relative concentrations secreted from individual granules were observed after incubation with 5-hydroxytryptamine, histamine, and tryptophan. In contrast, an alteration in individual cell content after such incubations, analyzed with capillary chromatography, was only found after incubation with 5-hydroxytryptamine. Cells incubated with 5-hydroxytryptamine or its precursor, tryptophan, released more 5-hydroxytryptamine and less histamine per secretory event relative to controls. Coincubation of the cells with pargyline and 5-hydroxytryptamine further reduced the release of histamine. Since cell content of histamine is unchanged, the reduction in its release must be due to its displacement to a nonreleasable compartment induced by 5-hydroxytryptamine granular uptake. Incubation with histamine increased histamine secretion and, surprisingly, also increased 5-hydroxytryptamine release without changing its cell content. This result is consistent with a relaxation of the storage matrix accompanying histamine granular uptake allowing more 5-hydroxytryptamine to be released. These results demonstrate that the intragranular mode of storage as well as granular uptake of biogenic amines affects the stoichiometry of their release.

Mast cells release the chemical messengers histamine and 5-hydroxytryptamine (5-HT) from secretory granules. Release occurs in an exocytotic manner similar to that in neurons and endocrine cells. Because they have large granules that can be monitored optically (*1*, *2*), mast cells have played an important role in the exploration of exocytotic mechanisms. Fusion of the granule with the plasma membrane exposes the interior vesicular matrix, composed of heparin sulfate and proteins (*3*), to the extracellular fluid. This causes a swelling of the matrix (*4*, *5*) that is accompanied by secretion of discrete packets of 5-HT (*6*) and histamine (*7*). Secretion of oxidizable biogenic amines can be observed at the single granule level with carbon fiber microelectrodes placed adjacent to the cell (*8*−*10*). Fast scan cyclic voltammetry, a technique in which the microelectrode potential is rapidly swept over a range of potentials (*11*), allows monitoring of multiple substances released from the same vesicle (*12*) such as histamine and 5-HT from mast cells (*7*). This technique provides the first opportunity to observe cosecretion during individual exocytotic events in real time. The combined use of capillary chromatography to measure the total content of individual cells (*7*, *13*, *14*) provides a way to examine the relative stoichiometry of release and content.

Studies of secretion from mast cells (*6*) and chromaffin cells (*15*, *16*) have revealed that release following membrane fusion has a time course that is much more complex than can be explained by simple diffusion of the contents out of the granule (*16*−*18*). Transient fusion events have been shown to release only a portion of the vesicular contents at a variety of cell types (*6*, *19*). Several reports have shown that the storage of the vesicular contents affects its subsequent release (*15*, *20*−*22*). Thus, the concept of a quantized nature of granular or vesicular release requires modification (*23*). A key finding in the evolution of the exocytotic concept was that substances are released in stoichiometric proportions to their original, stored form (*24*). The ability to monitor simultaneously two species contained within the granular matrix during individual exocytotic release events provides the unique opportunity to examine such stoichiometry at the single granule level, and test the quantal concept.

Histamine and 5-HT are taken up by mast cells (*25*−*30*) and packaged into granules by an active transport mechanism (*31*). There, they are associated with the matrix (*3*). They are costored in the same granules, and, during individual release events, the extracellular concentrations of each show a linear, positive correlation with each other (*32*). In this work, we have used the uptake processes to manipulate the cell content and storage of histamine and 5-HT to examine whether this affects the stoichiometry of their release. Our measurements of individual secretory events reveal that dramatic changes in release can occur.

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MATERIALS AND METHODS

Electrochemistry. Carbon-fiber disk electrodes for measurement of histamine and 5-HT release from cells were constructed as previously described (*11*). A 10 μm diameter carbon fiber (Thornel P-55, Amoco Corp. Greenville, SC) was sealed in a glass capillary with approximately 1 μm of glass surrounding the carbon fiber at the tip. The tips were polished at a 45° angle on a micropipet beveling wheel (K. T. Brown Type, Sutter Instrument Co., Novato, CA). Electrodes were electrochemically pretreated before use by application of a triangle potential waveform from +100 to +1400 to +100 mV at 800 V/s every 30 ms in 0.1 M NaOH for 15 s.

Electrodes were calibrated in a flow-injection apparatus (*7*). Buffer used for flow-injection analysis consisted of 12.5 mM Tris/HCl (pH 7.4) and 150 mM NaCl. The electrodes were used with an EI-400 potentiostat (Ensman Instrumentation, Bloomington, IN) in the two-electrode mode, and measurements were made with respect to a sodium-saturated calomel reference electrode (SSCE). The current response was filtered at 5 kHz and digitized with a 12-bit analog to digital converter (Labmaster DMA, Scientific Solutions, Solon, OH) interfaced to an IBM compatible personal computer with commercially available software (CV6, Ensman Instrumentation).

Cell Isolation. Mast cells were isolated as previously described (*7*) from male Sprague−Dawley rats (350−550 g). The cells were plated onto plastic cell-culture dishes (Falcon 3001, Becton Dickinson, Lincoln Park, NJ) and placed in a 37 °C incubator for at least 1 h before use to allow the cells to adhere to the culture dishes. Release measurements were made at room temperature on the day that the cells were isolated. The pH 7.4 physiological buffer in which cells were incubated and release measurements were conducted was composed of 12.5 mM Tris/HCl, 150 mM NaCl, 4.2 mM KCl, 5.6 mM glucose, 1.5 mM $CaCl_2$, and 1.4 mM $MgCl_2$.

Release Experiments. To measure release from individual granules, a culture plate containing mast cells was placed on the stage of an inverting microscope (Axiovert 35, Zeiss, Eastern Microscope, Raleigh, NC). A piezoelectric micropositioner (Kopf Model 640, Tujunga, CA) was used to position a carbon-fiber microelectrode next to a single cell. The electrode was brought into contact with the cell until the cell was slightly indented, and then the electrode was retracted 1 μm. Cyclic voltammograms at the cells employed a scan rate of 800 V/s and were repeated at 16.7 ms intervals. The potential range employed was sufficient to simultaneously oxidize histamine and 5-HT. A new microelectrode was used to take measurements at each cell.

A pressure ejection device (Picospritzer, General Valve Corp., Fairfield, NJ) was used (0.7 bar for 6 s) to introduce buffer solution with 5 μM calcium ionophore A23187 from a micropipet with a 10 μm inner-diameter tip. For release measurements with prior incubations, the cells were first incubated in physiological buffer for 1 h. The buffer was then removed from the culture plate and replaced with buffer containing the species to be loaded into the cells. The culture plate was returned to the incubator for 1 h in this solution. Before release studies, the culture plate was washed several times with the physiological buffer in which the experiments were to be performed.

Data Analysis. Cyclic voltammograms were background-subtracted with voltammograms collected immediately before stimulation of the cell or exposure of the surface to oxidizable substance. Concentration changes of 5-HT were determined from the average current collected in the 100 mV range around the peak oxidation potential for this wave. Similarly, concentration changes of histamine were constructed from the oxidation current at its peak potential for oxidation. Oxidation currents were converted to concentrations based on the average of pre- and postcalibrations. Widths of the spikes at half-height ($t_{1/2}$) and maximal concentrations of individual spikes (C_{max}) were obtained with locally written software (*17*). Each measured value is reported as the mean ± the standard error of the mean.

Chromatography Experiments. The chromatographic system used for the measurement of cell contents has been described previously (*7*). A commercial pump (Model LC-600, Shimadzu Corp., Kyoto, Japan) delivered a flow rate of 0.08 mL/min, and matched inner-diameter splitter capillaries were used so that the flow rate through the packed capillary column was about 60 nL/min. A cylindrical (1 mm length, 8 μm diameter) carbon fiber electrode was used as a detector. Current was measured with a model 427 current amplifier (Keithley Instrument Inc., Cleveland, OH) connected to a 16-bit analog to digital converter and IBM compatible computer. The time constant of the current amplifier was 300 ms and the data collection rate was 1 point/s. Histamine and dihydroxybenzylamine (internal standard, DHBA) were detected at an oxidation potential of +1300 mV vs Ag/AgCl. Shortly after elution of DHBA, the voltage was switched to +800 mV vs Ag/AgCl to enable the gain on the current amplifier to be increased by 1 order of magnitude for 5-HT and 5-hydroxytryptophan detection. The voltage change also improved selectivity for these two species because fewer species are oxidized at +800 mV than at +1300 mV.

For analysis of cell content, single cells were removed and transferred to 300 nL microvials with a micropipet of 10 μm inner diameter as previously described (*33*). Exactly 20.0 nL of internal standard which consisted of 10 μM DHBA in 0.3 N $HClO_4$ was added to each microvial with a specially constructed microdispenser pipet (*34*). The capped microvials were centrifuged at 12000*g* for 8 min and the supernatant from individual vials was injected directly into the chromatography column with a microinjector pipet.

Reagents. All compounds were obtained from Sigma (St. Louis, MO). Solutions of histamine were prepared by dilution of 50 μM stock solutions in 0.1 N $HClO_4$ and those of 5-HT were similarly prepared from a stock solution of 2.5 mM. Solutions were prepared in distilled deionized water. For chromatography experiments, the mobile phase was prepared by dilution of 85% phosphoric acid to 75 mM and addition of 0.3 mM hexane sulfonic acid. The pH was adjusted to 3.0 with NaOH and the solution was filtered through a 0.45 μm Nylon filter (Alltech, Deerfield, IL).

RESULTS

Voltammetry of Small Molecules Present in Mast Cells. Figure 1 shows background-subtracted fast-scan cyclic

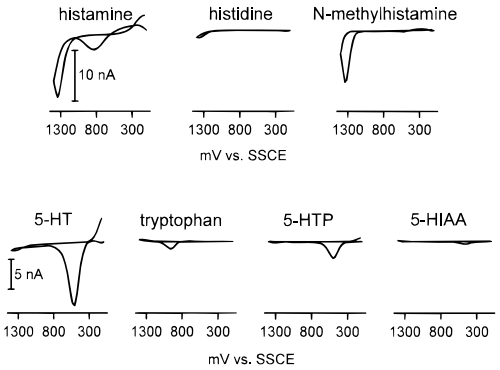

FIGURE 1: Cyclic voltammetry of various electroactive compounds present in mast cells in physiological buffer. Recordings made with a disk-shaped carbon fiber microelectrode at a scan rate of 800 V/s. The concentrations were 20 μM histamine, 20 μM histidine, 20 μM *N*-methylhistamine, 1 μM 5-HT, 1 μM tryptophan, 1 μM 5-hydroxytryptophan (5-HTP), and 1 μM 5-hydroxyindoleacetic acid (5-HIAA).

voltammograms obtained in physiological buffer of several electroactive species that are present in mast cells. The voltammogram of histamine (Figure 1) is characterized by a peak at about 1400 mV preceded by a smaller peak at about 800 mV. Although the amplitude of the feature at 800 mV is time dependent and unusable for quantification (*7*), it aids in histamine identification. The voltammogram of histidine, the synthetic precursor of histamine (Figure 1), is similar except the peak has a smaller amplitude because it does not adsorb as strongly to the electrode. The voltammogram of *N*-methylhistamine, histamine's major metabolite, is also similar to that of histamine except that no peak occurs at 800 mV. The peak for 5-HT oxidation occurs at much less positive potentials (500 mV, Figure 1). This technique is very sensitive for 5-HT since it strongly adsorbs at carbon surfaces (*35*). Tryptophan (Figure 1), the amino acid precursor of 5-HT, is oxidized at about 1000 mV. 5-Hydroxytryptophan, an intermediate in 5-HT synthesis, and 5-hydroxyindoleacetic acid, its major metabolite (5-HTP and 5-HIAA, Figure 1), are oxidized at the same potential as 5-HT. However, 5-HIAA does not adsorb and can only be detected at high concentrations. Except for 5-hydroxytryptophan and 5-HT, the unique features of the seven voltammograms in Figure 1 make all of these compounds distinguishable by fast-scan cyclic voltammetry.

Release at Mast Cells. Concentration traces obtained by cyclic voltammetry during release from freshly isolated mast cells incubated in physiological buffer are shown in the upper traces of Figures 2 and 3 for 5-HT and histamine, respectively (indicated as control). These traces were recorded simultaneously at two different potentials during cyclic voltammograms obtained at 16.7 ms intervals. The information for the instantaneous 5-HT concentration was obtained from the oxidation current at 500 mV in each cyclic voltammogram, and the concentration of histamine was obtained from the current at 1400 mV. Release is not observed until exposure of the cell to A23187, a calcium ionophore. Following a short delay, entry of Ca^{2+} into the cell evokes exocytosis of the intracellular granules resulting in concentration spikes recorded in the cyclic voltammograms.

As previously described (*7*), most concentration spikes consist of secretion of both substances as evidenced by coincident events at 500 and 1400 mV in the cyclic voltammograms. This is particularly apparent for the spikes marked with boxes in the top traces of Figure 2 and Figure 3. The mean maximal concentration (C_{max}) of 5-HT during each secretory spike was found to be 31 $\pm$ 1 nM, while that for histamine was 2.27 $\pm$ 0.04 μM (results from 64 freshly isolated cells that yielded a total of 5149 spikes). Thus, the concentration of 5-HT released is only 1.4% of the concentration of histamine. The widths of the spikes at half-height ($t_{1/2}$) were found to be similar for the two species (138 $\pm$ 1 ms and 125 $\pm$ 1 ms for 5-HT and histamine spikes, respectively). In all cells, secretion was monitored until it ceased.

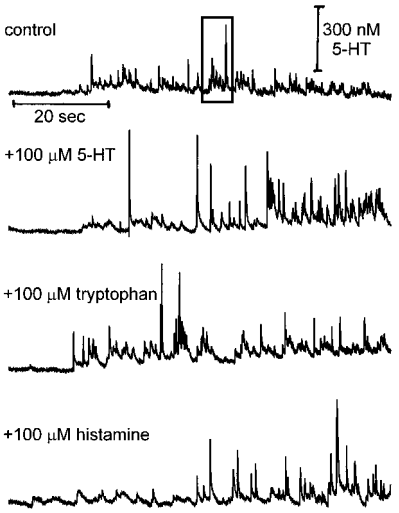

FIGURE 2: Concentration traces for 5-HT obtained from repetitive cyclic voltammograms obtained at mast cells stimulated with calcium ionophore A23187. Current at 500 mV for 5-HT oxidation was extracted from successive voltammograms and converted to concentration based on calibration. Each current spike corresponds to the release of an individual granule from the cell. Except for the control, all cells were incubated in physiological buffer with the indicated species for 1 h at 37 °C. The identical region marked by the box is also marked in Figure 3 so that the similarities in 5-HT and histamine release can be compared.

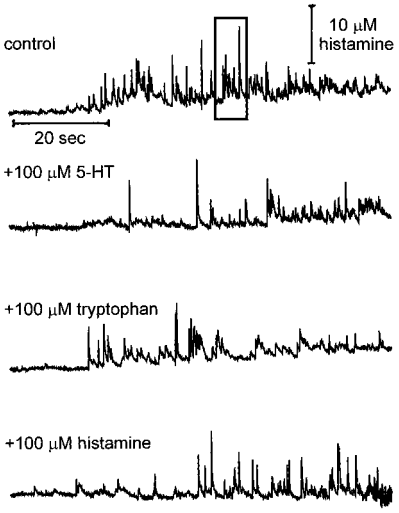

FIGURE 3: Concentration traces for histamine obtained from repetitive cyclic voltammograms obtained at mast cells stimulated with calcium ionophore A23187. The recordings are from the same voltammograms as in Figure 2 except the current at 1400 mV for histamine oxidation was extracted. Conversion to concentration based on calibration. Prior incubations as described in Figure 2.

Table 1: Measurement of Contents of Individual Mast Cells Using Capillary Chromatography

	cells	5-hydroxytryptophan (fmol)	5-HT (fmol)	histamine (fmol)	histidine (fmol)
control	12	0.4 ± 0.3	4 ± 1^b	150 ± 18^b	22 ± 9^b
100 μM 5-HT incubation	17	4 ± 4	16 ± 3^c	151 ± 13	14 ± 5
100 μM histamine incubation	16	0.8 ± 0.4	5.0 ± 0.9	109 ± 15	18 ± 7

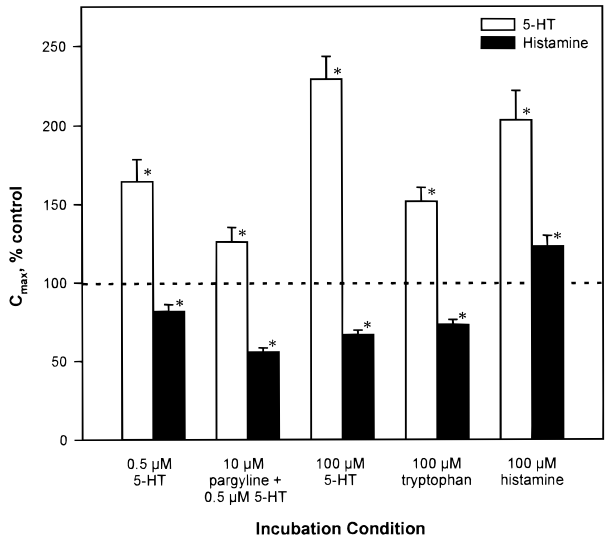

FIGURE 4: Maximal concentration (C_{max}) per exocytotic spike for histamine and 5-HT secretion from individual cells expressed as a percent of the release from freshly isolated cells (control). For the control set 5149 spikes were measured from 64 cells. For incubation with 0.5 μM 5-HT, 1094 spikes from 12 cells were measured. For incubation with 0.5 μM 5-HT and 10 μM pargyline, 1262 spikes from 14 cells were measured. For incubation with 100 μM 5-HT, 1926 spikes from 21 cells were measured. For incubation with 100 μM tryptophan, 1943 spikes from 22 cells were measured. For incubation with 100 μM histamine, 884 spikes from 11 cells were measured. (*) Means are significantly different from the control (95% confidence level, Student's *t*-test). Error bars correspond to sem.

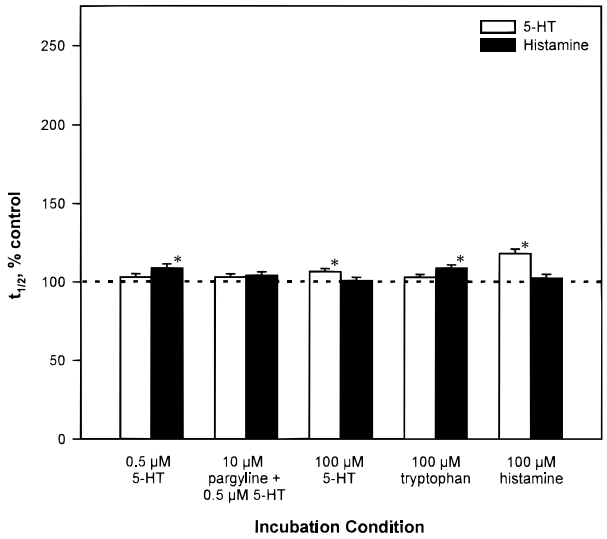

FIGURE 5: Width of spikes at half-height ($t_{1/2}$) per exocytotic event for histamine and 5-HT secretion from individual cells expressed as a percent of the release from freshly isolated cells (control). Data are from the same spikes as in Figure 4. (*) Means are significantly different from the control (95% confidence level, Student's *t*-test). Error bars correspond to sem.

In separate cells, the chemical composition was determined by capillary chromatography (Table 1). In addition to 5-HT and histamine, the amounts of 5-hydroxytryptophan and histidine, precursors of the two chemical messengers, were determined. As we have previously noted (*7*) the percentage of the cell content of 5-HT relative to histamine (2.7%) is higher than the percentage of the 5-HT concentration observed during secretion.

Effects of Incubation with 5-HT or Tryptophan. The remaining traces in Figures 2 and 3 were obtained in physiological buffer at cells that had been incubated at 37 °C with the indicated solutions. Prior incubation with 0.5 μM 5-HT resulted in 5-HT spikes that were greater in amplitude (summarized results from multiple cells, Figure 4). In contrast, the mean width of the 5-HT spikes at half-height was not significantly affected (Figure 5). Uptake of 5-HT by mast cells occurs by active transport (*25*, *30*, *36*, *37*) and its subsequent accumulation into granules is a saturable process with a K_M of 1.6 μM (*31*). Consistent with this, incubation with a much higher 5-HT concentration (100 μM, Figure 2) gave only a modest increase in the amount of 5-HT released, but in this case the spike widths were increased to a small but significant degree. Surprisingly, both of these incubations resulted in a significant reduction in the observed maximal histamine concentration for individual exocytotic events (Figure 3, pooled data in Figure 4), accompanied by small, but in some cases significant, increases in the mean spike width (Figure 5). Cells incubated with 100 μM 5-HT showed an increase in 5-HT content (Table 1). However, the histamine content was not altered. Thus, the lowered release of histamine observed with such incubations was not due to a lowering of cell content.

Incubation with 10 μM pargyline, a monoamine oxidase inhibitor, did not alter the characteristics of the exocytotic spikes (data not shown). However, prior incubation with both 0.5 μM 5-HT and 10 μM pargyline increased 5-HT release and further decreased the mean maximal concentration of histamine in the release events (Figure 4), with no significant effect on spike width for either type (Figure 5). Incubations with 100 μM tryptophan (Figure 2) also increased the mean spike amplitude for 5-HT, but similarly decreased that for histamine (Figure 3, data from multiple cells in Figure 4). 5-HT can be formed from tryptophan in mast cells due to hydroxylation to form 5-hydroxytryptophan followed by decarboxylation (*38*).

Effects of Incubation with Histamine. Release was also examined following incubation with histamine. Histamine accumulates in mast cells by a rather inefficient manner with an apparent K_m of 2 mM (*37*). Histamine can enter the granules via a saturable process with a K_m of 91 μM (*31*). Incubation with histamine did not significantly alter cell content of any species examined, although the content of histamine appeared to decrease slightly. The variability in cell content made this difficult to establish. However,

following incubation spikes during secretion exhibited a greater maximal histamine concentration than control cells (Figure 3, data from composite cells in Figure 4), but with no affect on mean histamine spike width (Figure 5). Surprisingly, this incubation also increased the mean 5-HT concentration for each spike (Figure 2 and Figure 4) as well as its width at half-height (Figure 5).

DISCUSSION

In this work, we have used two microanalytical tools, capillary chromatography and microelectrodes, to examine the interplay between uptake, storage, and release of 5-HT and histamine in mast cells. Capillary chromatography allows individual cell content to be determined while microelectrodes permit simultaneous detection of 5-HT and histamine corelease from individual granules. Combined, the data allow a more thorough characterization of the interaction of the chemical messengers in the intracellular granules. The results show that only 5-HT incubations significantly altered the content of individual cells. However, the concentrations of the two amines released from granules varied dramatically with all the substances tested. This result leads to the surprising conclusion that cells do not necessarily release chemical messengers in amounts proportional to what they contain. This is in contrast to the conclusion we reached at adrenal medullary cells where, with the use of the same techniques, we found that the ratio of release of epinephrine and norepinephrine was similar to the cell content (*39*). However, in that study, we did not attempt to alter the cell contents as in this work. Since the primary site of sequestration of 5-HT and histamine is in the secretory granules, these results suggest that the mode of storage in the granules can affect the release process.

This mechanism of storage of amines in mast cell granules allows for their long-term sequestration. Indeed, in the absence of stimulation, turnover of histamine and 5-HT in mast cells is very slow with a half-life of greater than 20 days (*29*). The intragranular contents can be isolated as an intact matrix that has the properties of a charged polymer network (*2*). Histamine is strongly associated with the granular matrix as demonstrated by nuclear magnetic resonance (*40*). It is a dication at the pH of the intragranular matrix (pH = 6) and, thus, has a greater propensity to cross-link the matrix than 5-HT, which is a monocation at this pH. The association of both molecules with the matrix is quite strong since turnover is slow and the concentration of their metabolites inside the cell is low (*27, 29*). Furthermore, high concentrations of both 5-HT and histamine cause the granular matrix to condense (*1*). Consistent with costorage, either can displace the other in granules (*29, 41*). The release of these compounds from the granule matrix has many of the properties of an ion exchange process (*2*).

The time course of the individual secretory events provides immediate evidence that the intragranular storage mechanism dramatically affects release of the granule contents. The mean duration of the secretory events (width at half-height > 120 ms) is much longer than those recorded by cyclic voltammetry for catecholamine secretion from chromaffin cell vesicles (70 ms) (*12*). Furthermore, the times exceed by approximately 10-fold those predicted for cyclic voltammetric detection of compounds released from a spherical vesicle in which the molecules can exit by free diffusion (*17*). As we have previously shown at chromaffin cells, the lengthy duration of extrusion of the cell contents is due to the strong association of the granule contents (*16*, *20*, *22*). Upon cell-granule fusion, the granule contents are exposed to the extracellular pH of 7.4, a condition that causes matrix swelling by about 40% (*1*). This swelling is accompanied by the concomitant release of 5-HT and histamine. Thus, the slow rate of 5-HT and histamine release from mast cell granules is a consequence of this process at these cells as well.

Incubation of the cells with substances known to be accumulated in the granules provides the opportunity to alter the vesicular contents. Amine accumulation in granules involves two transporters, one at the plasma membrane and the other at the granule. The granule transporter has characteristics similar to that for the vesicular monoamine transporter in neurons although the precise transporter in these cells has not yet been identified (*42*). These transporters are reversible, and one amine can serve as an inhibitor of the transport of the other (*31*). Following incubation of mast cells with 5-HT or tryptophan, its biosynthetic precursor, our results show that the releasable granular concentration of 5-HT is increased, consistent with the high affinity of the granular transporter for 5-HT. Note, however, that whereas a 4-fold increase in 5-HT cell content was obtained following incubation with 100 μM 5-HT, the average maximal concentration during each exocytotic event increased by less than 3-fold. The high affinity of the granule transporter ensures that most 5-HT is stored in granules. Therefore, this further suggests that not all the messenger is released upon exocytosis, although the data suggest the majority is.

The average histamine concentration released from individual granules was significantly lowered by incubations with 5-HT or tryptophan whereas cell content was unchanged. The reduced relative release of histamine seen here at the granular level is similar to the reduced release of histamine observed from populations of mast cells following similar manipulations (*43*, *44*). This has been attributed either to selective transport of 5-HT into cytosolic vesicles followed by its preferential release (*43*, *45*), or post-secretory uptake of 5-HT (*44*). The results shown here are inconsistent with either of these proposals. The cosecretion of both 5-HT and histamine continues under all conditions indicating that release in all cases is from the same granule compartment. Furthermore, since the half width of 5-HT spikes never decreased with any of the conditions tested, uptake is not affected by any of the conditions employed on the time scale of the individual exocytotic events. Rather, since the histamine cell content is unchanged, the differential release of 5-HT and histamine must have its origin in the granular compartment.

Influx of 5-HT into the granule via the transporter can have dramatic consequences. It can alter the organization and size of the granule matrixes (*1*) which could place intragranular histamine in an environment where it has greater difficulty leaving the granule matrix after exocytosis because it is more tightly associated with the granular matrix. More likely, since 5-HT accumulation in granules occurs at the expense of histamine (*29*) and it can block the subsequent reuptake of histamine into granules (*31*), histamine could

be displaced into the cytoplasmic compartment where it is unavailable for release. The further decrease in histamine release caused by 5-HT incubation with pargyline is consistent with both of these possibilities since protection from monoamine oxidase would enable 5-HT multiple opportunities to enter and exit the granule and thus affect its contents. However, it is unclear why 5-HT incubation did not cause a greater increase in its own release.

Incubation with histamine did not affect cell histamine or 5-HT content. However, prior studies with radiolabeled compounds have demonstrated that histamine is accumulated in cells under similar conditions (*26*). Thus, our failure to observe a change in content may arise because the uptake of exogenous material is accompanied by displacement of endogenous material with a net result that cell content is unchanged. Consistent with an alteration of histamine storage following such incubations, the concentration of histamine released for each exocytotic event was found to increase.

The more remarkable result is that incubation with histamine leads to a pronounced increase in 5-HT release per exocytotic event. This occurs without a change in its cell content or the time course of the concentration spikes. Thus, the increased release must reflect a movement of previously nonreleasable 5-HT to a granular location from which it can be secreted. The high affinity of the vesicular transporter will maintain cytoplasmic 5-HT at low levels, making this an unlikely source. Another possibility, the transfer of 5-HT from storage to releasable granules, also appears unlikely since the measurements reflect the concentration of all exocytotic events, and most mast cells granules exocytose with the stimulation employed. A more likely possibility is that uptake of exogenous histamine causes displacement of endogenous histamine inside the granule. Since stored histamine cross-links the granular matrix due to its divalent character, such displacement would lead to a rearrangement in the intragranular association, and could loosen the association of 5-HT, allowing its increased release. This could occur without a change in time course of the spikes since 5-HT is only a minor component of the vesicle. Furthermore, increased release without a decrease in half-width has precedent: exocytotic spikes of catecholamine from chromaffin cells are larger and actually have greater half widths when released into basic medium, a condition that promotes expansion of the vesicle matrix (*15*).

Thus, the data shown here indicate that the granule matrix normally restricts release so that only a partial quantity of the granule contents is secreted on the time scale of a secretory spike. This has been observed at chromaffin cells, as described above, and was also proposed earlier by us to explain the different ratios of histamine to 5-HT release from single exocytotic events when compared to cell content (*7*). Indeed, the ratio of released 5-HT to histamine following histamine incubation is closer to the ratio of their respective cell contents. Thus, restricted efflux from a gel matrix joins flickering of the newly formed cell-vesicle fusion pore (*6*) as possible mechanisms that can lead to partial, nonquantized release following exocytosis.

The results of this study support previous reports that differential release of cell contents can occur from mast cells, and provide a possible mechanism which accounts for such observations. These measurements at the level of individual exocytotic events demonstrate that the differential release can occur as a consequence of changes at the level of individual granules. The results show that release can be altered both as a consequence of uptake into granules and intragranular storage. The nonstoichiometric ratio of release and cell content seen following 5-HT and histamine incubations is surprising and a result quite different from that reached in chromaffin cells which appear to release their granule contents in stoichiometric proportions (*24*). It will be of interest to see if the plasticity in secreted amounts can occur in other types of secretory cells such as neurons (*23*). Indeed, we have already shown that under appropriate conditions dopamine neurons in vivo can be forced to secrete 5-HT (*35*).

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