Degranulation of Individual Mast Cells in Response to Ca^{2+} and Guanine Nucleotides: An All-or-None Event

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Abstract. Widespread experience indicates that application of suboptimal concentrations of stimulating ligands (secretagogues) to secretory cells elicits submaximal extents of secretion. Similarly, for permeabilized secretory cells, the extent of secretion is related to the concentration of applied intracellular effectors. We investigated the relationship between the extent of secretion from mast cells (assessed as the release of hexosaminidase) and the degranulation (exocytosis) responses of individual cells. For permeabilized mast cells stimulated by the effector combination Ca^{2+} plus GTP-γ-S and for intact cells stimulated by the Ca^{2+} ionophore ionomycin, we found that exocytosis has the characteristics of an all-or-none process at the level of the individual cell. With a suboptimal stimulus, the population comprised only totally degranulated cells and fully replete cells. In contrast, a suboptimal concentration of compound 48/80 applied to intact cells induced a partial degree of degranulation. This was determined by observing the morphological changes accompanying degranulation by light and electron microscopy and also as a reduction in the intensity of light scattered at 90°, indicative of a change in the cell-refractive index. These results may be explained by the existence of a threshold sensitivity to the combined effectors that is set at the level of individual cells and not at the granule level.

We used flow cytometry to establish the relationship between the extent of degranulation in individual rat peritoneal mast cells and the extent of secretion in the population (measured as the percentage release of total hexosaminidase). For comparison, secretion was also elicited by applying the Ca^{2+} ionophore ionomycin or compound 48/80 to intact cells. For permeabilized cells and also for intact cells stimulated with the ionophore, levels of stimulation that generate partial secretion gave rise to bimodal frequency distributions of 90° light scatter. In contrast, a partial stimulus to secretion by compound 48/80 resulted in a single population of partially degranulated cells, the degree of degranulation varying across the cell population. The difference between the all-or-none responses of the permeabilized or ionophore-treated cells and the graded responses of cells activated by compound 48/80 is likely to stem from differences in the effective calcium stimulus. Whereas cells stimulated with receptor-directed agonists can undergo transient and localized Ca^{2+} changes, a homogeneous and persistent stimulus is sensed at every potential exocytotic site in the permeabilized cells.

OUR understanding of stimulated secretion in terms of intracellular signals and their target proteins leaves an important question unanswered. How can cells that are able to release close to 100% of their granule contents, such as mast cells, give rise to partial secretion? Does this reflect a difference in the susceptibility of individual secretory granules to undergo exocytosis, or is it a consequence of inhomogeneities in the intracellular signals or their targets?

The classic experiment in the study of secretory processes involves the application of graded stimuli to a tissue or a population of susceptible cells and the measurement of released secretory products. Typically the data are presented as dose–response curves in which a suboptimal stimulus to secretion causes a partial response. Unlike a simple enzyme-catalyzed process in which the concentration of substrate and other modulatory effectors dictate the rate but not the final extent of reaction, in secretion, the rate of release may be relatively independent of these factors (16, 31). Basic to this difference is the fact that secretion comprises a sequence of unitary events in which individual granules fuse with the plasma membrane independently of each other, in an all-or-

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none manner. A partial extent of secretion indicates that only a fraction of the total number of secretory granules in the experimental sample has undergone exocytosis. For example, a rat mast cell contains ~1,000 granules and a typical measurement might involve ~1,000 cells per sample.

The exocytotic fusion reaction has become amenable to experimental investigation with the development of techniques of plasma membrane permeabilization. These permit the composition of the cytosol to be modified by introduction of membrane-impermeant solutes such as effectors that can directly activate exocytosis and allow the early events in the pathway to be bypassed (24, 33). To understand the mechanism, it is important to determine the sensitivity of the system to different effector concentrations. Again, it is common to apply graded stimuli (such as Ca^{2+} buffers and guanine nucleotides) and measure the consequent extent of release from a cell population. Simple concentration ("dose")–response relationships based on the average performance of large numbers of cells reveal nothing, however, about the responses of individual cells. It is consequently impossible to tell whether the extent of secretion reflects the extent of binding and hence the affinity of the effectors for specific intracellular binding proteins, or reflects a threshold sensitivity of individual exocytotic units; these could be either the secretory granules or the cells.

In this paper we show that for permeabilized and Ca^{2+} ionophore–stimulated intact mast cells, the extent of secretion in response to a suboptimal stimulus is set by a threshold sensitivity that is determined at the cellular level and not at the level of the secretory granules. Degranulation of the cells is an all-or-none phenomenon. On the other hand, for a suboptimal stimulus delivered by a cell surface–directed ligand such as compound 48/80 (a polycationic condensation product of *N*-methyl-*p*-methoxy phenylethylamine with formaldehyde [1, 19] considered to act as a receptor-mimetic agonist), the extent of exocytosis by individual cells is partial.

Materials and Methods

Cell Preparation

Mast cells were obtained by peritoneal lavage of large (>300 g) Sprague–Dawley rats. The mast cells were isolated from contaminating cell types by centrifugation through a cushion of Percoll (Pharmacia Ltd, St. Albans, Herts, UK) as previously described (32), washed twice by resuspension and centrifugation, and finally suspended in a buffered electrolyte solution prepared from analytical-grade salts (BDH analar grade) and which comprised 137 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 20 mM Pipes and 1 mg/ml BSA (pH 6.8). For experiments with intact cells, glucose (1 mg/ml) was added.

Secretion from Cell Suspensions

Before permeabilization, cells were incubated at 37°C for 5 min with metabolic inhibitors (2-deoxyglucose [6 mM] and antimycin-A [5 μM]) to deplete intracellular ATP. They were then permeabilized (at a final cell density of 3.3×10^5/ml) with 0.4 IU/ml streptolysin-O (SL-O)[1], in the presence of 1 mM MgATP and 3 mM CaEGTA buffer to regulate Ca^{2+} in the range of 10^{-8}–10^{-5} M (pCa 8 to pCa 5) (12), and guanosine 5′-[γ-thio]-triphosphate (GTP-γ-S) at the indicated concentrations. After incubation for 10 min at 37°C, the cells were quenched by addition of 0.5 ml of ice-cold buffer supplemented with 5 mM EDTA. Alternatively, for time course measurements (see Figs. 1 and 2), samples of cells were transferred at various times from bulk incubations into the quenching medium as previously described (31). For stimulation of intact cells (by ionomycin and compound 48/80), the preincubation with metabolic inhibitors was omitted and the incubation medium was supplemented with $CaCl_2$ (1 mM) and glucose (5.6 mM). Incubation was for 15 min. After centrifugation, the supernatants were sampled for the measurement of released *N*-acetyl-β-D-glucosaminidase (hexosaminidase) as previously described (12) and the cells were resuspended and kept on ice until analyzed by flow cytometry. On occasion, they were also fixed by addition of paraformaldehyde to a final concentration of 1%.

Preparation of Cells for Electron Microscopy

0.2-ml aliquots of cell suspension were placed on plastic coverslips (Thermanox; Life Sciences Technology, Paisley, UK) and the cells allowed to adhere for 30 min at room temperature before transferring to a 37°C incubator. The cells were then treated with metabolic inhibitors (concentrations as above), followed 5 min later by addition of a medium containing SL-O, MgATP, and Ca^{2+} buffer (final concentrations as above) and incubated at 37°C for a further 10 min. The supernatants were then aspirated and replaced by a fixing solution containing 1% glutaraldehyde (TAAB Laboratories, Reading, UK) made up in 0.15 M NaCl, 20 mM Hepes, 1 mM EGTA, 2 mM $MgCl_2$, pH 7.0. After 30 min the solution was changed to 1% OsO_4 in Na-cacodylate, pH 7.0; after a further 15 min, the cells were rinsed in PBS. The coverslips were taken through an acetone dehydration series (50, 70, 90, and 2 × 100%) and then critical-point dried with liquid CO_2 as the intermediate fluid. The coverslips were cut into strips (~3 × 5 mm) with scissors and fastened onto copper mounts with conductive glue and sputtered with gold. The cells were examined using a 100CX electron microscope (JEOL (UK) Ltd., London, UK) with high resolution scanning attachment.

Flow Cytometry

Suspensions of intact and permeabilized mast cells were analyzed using a cytometer/cell sorter (Epics Elite; Coulter Electronics, Ltd., Luton, UK). The cells were illuminated at 488 nm and the instrument was set to detect forward and 90° light scattering (side scatter) from each cell. The data collected are presented either as single-parameter histograms of log 90° scatter or as dual parameter plots of forward and log 90° scatter. A low level discriminator eliminated the contribution from very small particles and electrical noise. Each distribution shown represents data collected from at least 5,000 cells. Electronic cell sorting was used to select subpopulations from nonoverlapping regions of the 90° light-scatter histograms. We also conducted some analyses using a FACS Analyzer (Becton Dickinson, Mountain View, CA) that was set to collect (electronic) cell volume and 90° light-scatter signals. The data closely resembled those obtained with the Coulter instrument.

Results

Secretion from Permeabilized Cells

For suspensions of mast cells, the final extent of secretion depends on the strength of the stimulus, either the concentration of a receptor-directed ligand applied to intact cells or the concentration of intracellular effectors (Ca^{2+} and guanine nucleotides) applied to permeabilized cells. To relate the measured extent of secretion to the responses at the single-cell level, it is essential to ensure that completion has been attained. If it has not, then partial secretion determined after a fixed period of time could just be due to a slower rate of release. The time course of hexosaminidase release from SL-O–permeabilized mast cells is illustrated in Fig. 1. Cells were permeabilized in the presence of fixed concentrations of GTP-γ-S (20 μM) in tubes containing different Ca^{2+} concentrations (set with appropriate CaEGTA buffers). MgATP was also provided. Secretion, determined by timed sampling from each tube, proceeded over a period of ~3 min; final

1. *Abbreviations used in this paper:* GTP-γ-S, guanosine 5′[γ-thio]triphosphate; SL-O, streptolysin-O.

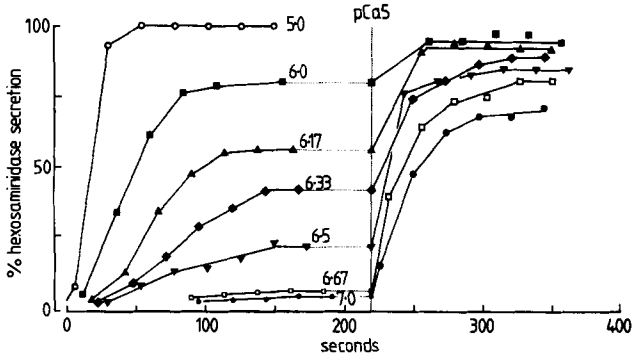

Figure 1. Progress of secretion in response to GTP-γ-S and a range of Ca^{2+} concentrations. Mast cells were permeabilized by SL-O in the presence of GTP-γ-S (10 μM), Mg-ATP (1 mM) and a range of Ca^{2+} buffers (1 mM EGTA) to set pCa as indicated. Samples were withdrawn and quenched at successive intervals. After allowing secretion to terminate (~3 min), a high concentration (5 mM EGTA) of Ca^{2+} buffer (*pCa5*) was added, and further timed samples were removed. Secretion was measured as released hexosaminidase and is expressed as the percentage of the amount liberated from cells lysed with 0.1% Triton X-100.

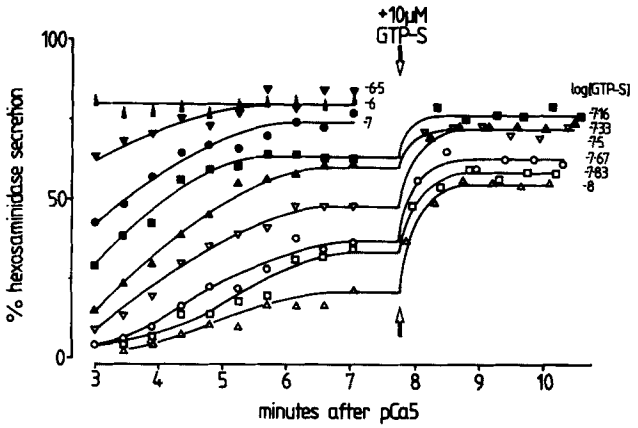

Figure 2. Progress of secretion in response to Ca^{2+} and a range of GTP-γ-S concentrations. Mast cells were permeabilized by SL-O in the presence of Ca^{2+} (pCa5) Mg-ATP, and a range of GTP-γ-S concentrations. Samples were withdrawn and quenched at the times indicated. After allowing secretion to terminate, a high concentration (*10 μM*) GTP-γ-S was added, and further timed samples were removed. Secretion was measured as released hexosaminidase. In this experiment, the onset of secretion was preceded by a delay. This is a characteristic of the presence of ATP and becomes more prolonged as the strength of the stimulus is diminished (>3 min for 10^{-8} M GTP-γ-S [31]).

levels at this time represent a typical dose-response for Ca^{2+} at these conditions. Approximately 50% release was achieved at pCa 6.17 (0.676 μM), indicating that ~50% of the total granules contained in the cell suspension had fused and released their contents. In the next stage of the experiment, the concentration of Ca^{2+} was elevated to pCa 5 (10 μM) in all the tubes. This had the effect of restarting secretion in those cells that had initially been subject to a suboptimal stimulus, continuing to levels not far short of those induced by an initial stimulus of 10 μM Ca^{2+}.

Fig. 2 illustrates the result of a similar experiment, but here the concentration of Ca^{2+} was fixed (at pCa 5) and the time course and extent of secretion due to different concentrations of GTP-γ-S was measured. Once again, secretion due to the partial stimulus terminated to give the characteristic dose-response; when the cells were subsequently supplemented with an optimal concentration (10 μM) of GTP-γ-S, secretion recommenced, terminating at a level approaching the maximum. These experiments show that a suboptimal stimulus elicits only a partial secretion from suspensions of permeabilized cells. However, when the level of stimulation is then further increased, another bout of release ensues, indicating that previously uninvolved exocytotic units (granules or cells) can be recruited into the secretory process.

These experiments define characteristics of cell suspensions representing the summation of all the individual cell responses; however, they reveal nothing about the levels of secretion attained by the individual cells. We have approached this problem in a number of different ways.

Electron Microscopy of Permeabilized and Intact Mast Cells

Using scanning EM we found that mast cells permeabilized at low Ca^{2+} (pCa 8) appear as broadly spherical with their surface showing numerous microvillous processes (Fig. 3 *A*). The permeabilization, which is sufficient to permit leakage of proteins such as lactate dehydrogenase (M_r = 140,000) (14), causes no visible damage to the integrity of the cell membrane when examined at this resolution. The appearance is somewhat altered from normal intact unstimulated cells, where cell surface folds are more prominent than microvilli (Fig. 3 *D*; and references 5, 27). The cause of this morphological change is not known, but a loss of G-actin after permeabilization will inevitably affect the cytoskeleton by perturbing the normal equilibrium between actin filaments and unpolymerized G-actin. It is worth noting that although experiments on permeabilized cells are usually interpreted in terms of the exocytotic fusion reaction, these cells have an altered surface morphology; secretion from intact cells may also require rearrangement of part of the cytoskeleton as part of the overall exocytotic process (18).

When permeabilized in the presence of GTP-γ-S (20 μM) and a concentration of Ca^{2+} sufficient to elicit maximal secretion, degranulation of the cells is plainly evident (Fig. 3 *B*), with exteriorized granules now adherent to the cell surface and the adjacent substratum. The microvilli largely disappear. After degranulation the cells seem to be very fragile, and despite taking precautions in all experiments, a proportion of the cells appeared to have been damaged so that only the underlying membrane and adjacent exteriorized granules were seen (data not shown).

When an intermediate stimulus is applied to the permeabilized cells, scanning EM shows that the cells take on the appearance of either the resting or the fully stimulated morphology. This is illustrated in Fig. 3 *C*, which shows two neighboring cells that had been subjected to stimulation by GTP-γ-S (20 μM) and Ca^{2+} (pCa 6.25). In contrast, with partial secretion due to compound 48/80, all cells appeared to have degranulated to at least some extent (Fig. 3 *E*).

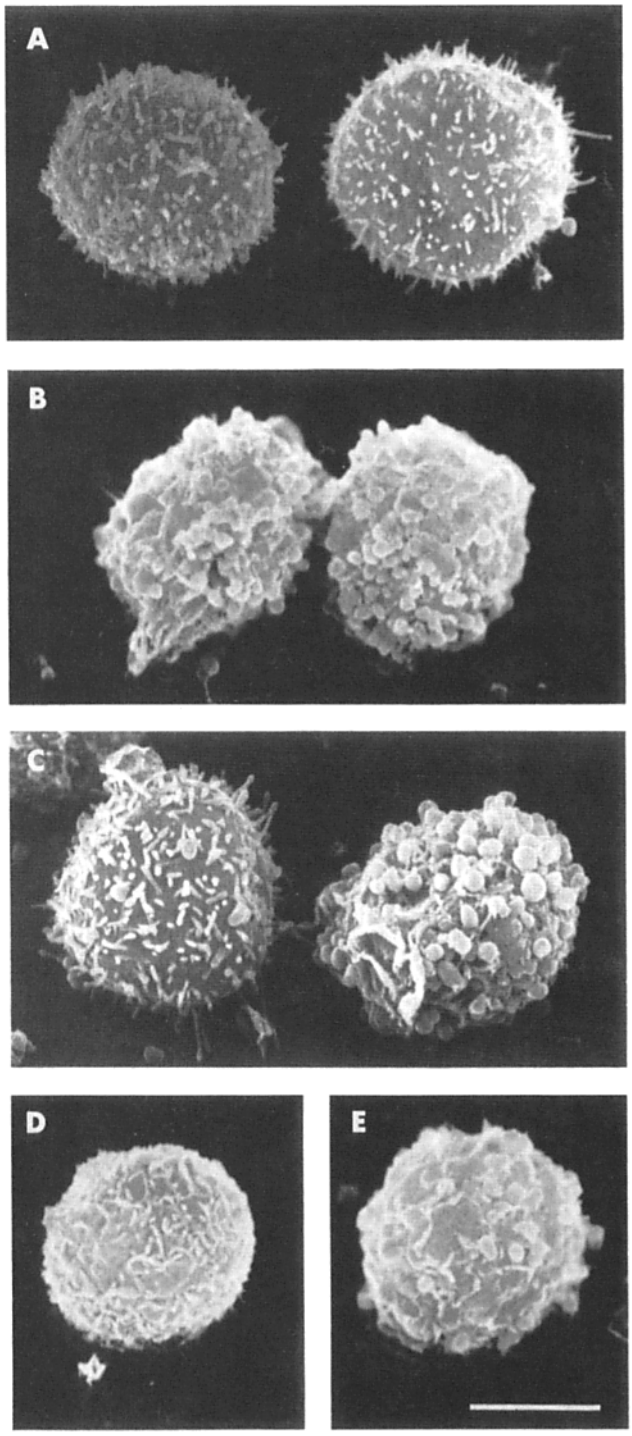

Figure 3. Scanning microscopy of permeabilized rat mast cells. The figure illustrates permeabilized and intact mast cells stimulated to undergo exocytosis to variable degrees. Permeabilized mast cells treated with GTP-γ-S and calcium buffer to regulate pCa8 (*A*), pCa5 (*B*), and pCa6.25 (*C*). *D* illustrates an intact unstimulated cell and *E* illustrates a cell presumed to be partially degranulated after treatment with a concentration of compound 48/80 (1 μg/ml), which induces ~50% secretion. Bar, 5 μm.

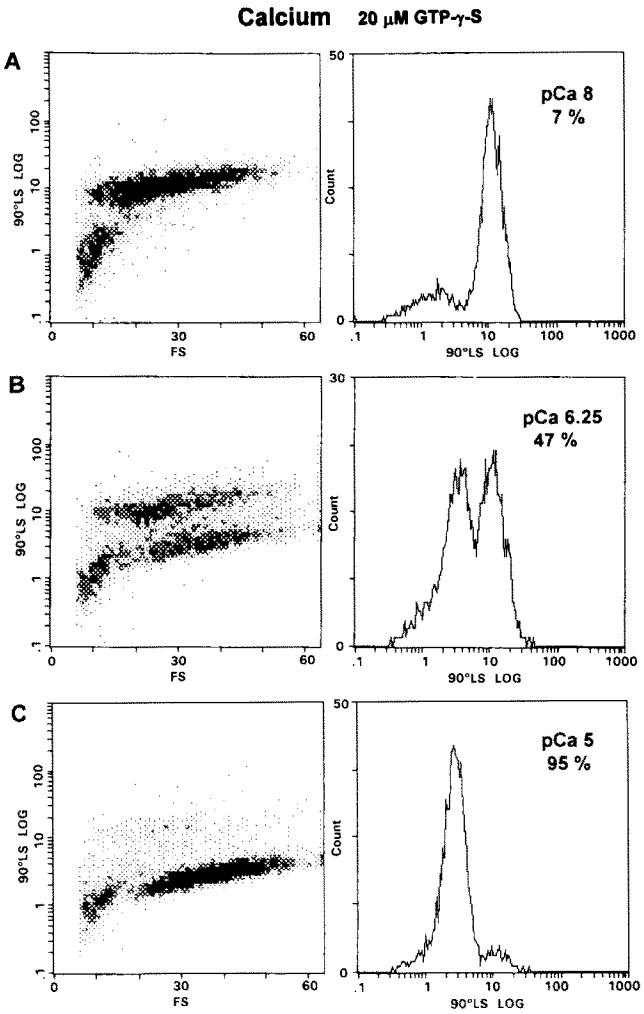

Figure 4. Flow-cytometric analysis of mast cells stimulated to secrete by three concentrations of Ca^{2+}. Mast cells were permeabilized by SL-O in the presence of Ca^{2+} buffers (3 mM EGTA, 1 mM Mg-ATP, and 20 μM GTP-γ-S). The lefthand panels show dual parameter dot plots (bivariate distributions) of 90° light scatter (log scale) versus forward light scatter (linear scale). The righthand panels show the corresponding single parameter histograms of 90° scatter (log scale). The Ca^{2+} level and extent of secretion are indicated in each case.

Flow Cytometry

Rat mast cells contain large numbers of secretory granules, which makes them highly refractile. This feature also manifests itself in the light-scattering properties of the cells, particularly at scattering angles around 90°. When the cells have undergone exocytosis, their refractility is lost and their ability to scatter light at 90° is correspondingly diminished. We have used this attribute to classify populations of permeabilized mast cells after partial or maximal stimulation in the manner described above. We have also analyzed populations of intact cells treated with the secretagogue compound 48/80 and the Ca^{2+}-ionophore ionomycin. To resolve the cell subpopulations present in these experiments, we have collected both forward and 90° light-scatter intensities from each cell. (The magnitude of the forward scatter signal is determined principally by cell size.)

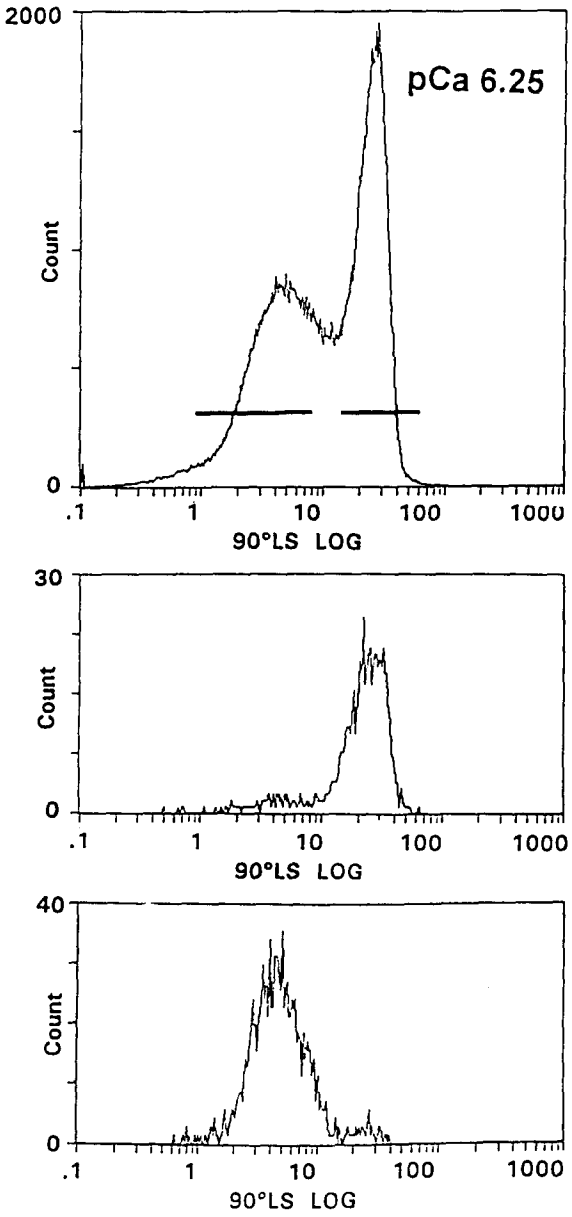

Figure 5. Analysis of sorted subpopulations of partially stimulated, permeabilized mast cells. Histograms of 90° scatter (log scale) of mast cells permeabilized in the presence of 20 μM GTP-γ-S at pCa6.25, producing 43% secretion. The top panel shows data obtained from cells before sorting; the sorting criteria are indicated as horizontal lines. The center panel shows the light-scatter distribution of cells sorted from the righthand peak of the top panel and then reanalyzed. The bottom panel shows the profile of cells sorted from the lefthand peak.

In the experiment illustrated in Fig. 4, mast cells permeabilized by SL-O in the presence of MgATP and GTP-γ-S (20 μM) were stimulated by three different concentrations of Ca^{2+} (Fig. 4, *A*, pCa 8; *B*, pCa 6.25; and *C*; pCa 5, set with 3 mM CaEGTA buffers). The panels on the left of Fig. 4 illustrate the bivariate distributions of the 90° and forward light-scatter intensities from individual cells, displayed as dot plots. Univariate histograms of log 90° scatter obtained from the same data are presented in the righthand panels of Fig. 4. All the intensities are relative. After minimal stimulation (Fig. 4 *A*, 8% hexosaminidase secretion), most of the cells form a single distinct population characterized by a broad range of forward scatter intensities (indicating a range of cell sizes) and a rather tightly defined range of 90° scatter intensities. After maximal stimulation (Fig. 4 *C*, 95% secretion), a similar unimodal pattern is generated, but now the principal population scatters less light at 90° whereas its median forward scatter intensity is slightly higher. This indicates that cells from a population that has undergone exocytosis scatter less light at 90° than those that have retained their granules. Also, the slightly enhanced forward scattering indicates that the cells are somewhat larger. (The subpopulation of particles evident even at pCa 8 at low scatter intensities, both forward and at 90° [between 1 and 10 on the log scale], consists of contaminating cells and subcellular debris.)

In contrast to these situations in which, by definition, almost all the cells are entirely replete or entirely degranulated, treatment with a partial stimulus (Fig. 4 *B*, pCa 6.25, 47% hexosaminidase secretion) revealed a bimodal scatter profile indicating two principal subpopulations. These encompass approximately equal areas, and on both types of display are situated exactly in the positions occupied by the maximally and minimally stimulated cells. Because the univariate histograms are equivalent to projections of the bivariate distributions on the 90° light-scatter axis, they tend to exaggerate the amount of overlap between the two subpopulations. This is because the profiles of each subpopulation in the dot plots are canted with respect to the horizontal axis. The dot plots in Fig. 4 show that the amount of overlap is in fact very small.

An alternative way of achieving partial stimulation of permeabilized mast cells is to fix a high Ca^{2+} and to vary the concentration of the guanine nucleotide. We tested the responses to both GTP-γ-S and GTP. The data are very similar to those of Fig. 4. From these experiments, we conclude that a stimulus resulting in release of 50% of the secretory product causes half of the cells to degranulate totally, leaving the remaining cells unaffected. This finding implies that the permeabilized cells respond to stimulation in an all-or-none manner.

Cell Sorting

To verify that partially stimulated, permeabilized mast cells consist only of completely degranulated and completely undegranulated cells, electronic cell sorting of the major subpopulations was performed. The morphology of the sorted cells was examined by light microscopy; then, to check the purity and stability of the sorted cells, they were run a second time through the instrument. For the experiment illustrated in Fig. 5, cells were stimulated by permeabilization in the presence of 20 μM GTP-γ-S at pCa 6.25, inducing 43% secretion. The top panel shows the 90° scatter distribution; the two sort criteria are indicated by horizontal bars. The lefthand (less intense) population, expected to be the degranulated cells, generated the data shown in the bottom panel; the righthand (brighter) population gave rise to the data shown in the center panel. These are expected to be undegranulated cells. The small subpopulation at lower intensity shown in this panel most likely corresponds to cells that have degranulated or been damaged by the sorting process.

Light microscopy of the sorted cells confirms these assign-

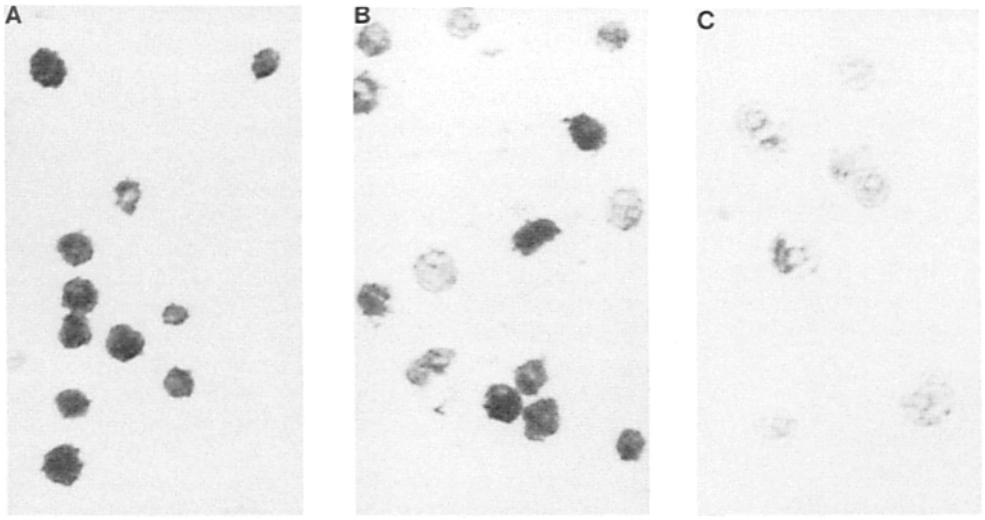

Figure 6. Light micrographs of mast cells stained with toluidine blue after partial stimulation and cell sorting. Contrast-enhanced micrographs of cells present in the populations depicted in Fig. 5: unsorted cells (*center*), cells sorted from the lefthand peak (*right*), and cells sorted from the righthand peak (*left*).

ments. Fig. 6 (center panel) shows a light micrograph of the cell preparation before analysis or sorting. All the preparations in this figure were treated with toluidine blue to reveal the presence of secretory granules. Both unstained (degranulated) and stained (undegranulated) cells are evident. The lefthand panel shows a micrograph of cells sorted from the brighter subpopulation and the righthand panel depicts cells sorted from the dimmer subpopulation. Nearly all cells taken from the brighter peak were deeply stained and retained the features of resting mast cells, whereas the cells from the lefthand (dimmer) peak appear as ghosts, devoid of granules, and confirming their assignment as degranulated cells.

Flow Cytometry of Intact Mast Cells

The scatter profile of a population of intact cells stimulated with compound 48/80 is characteristic of a very different relationship between secretion and degranulation (Fig. 7). As in previous experiments, the cells were subjected to levels of stimulation designed to induce zero, partial, and extensive release of granule contents. The actual levels of release obtained after a 15-min incubation were 8 (*A*), 28 (*B*), and 78% (*C*) (secretion due to compound 48/80 is rapid, going to completion within 10 s [10]). Unlike the permeabilized cells, each of these preparations gave rise to only a single major peak in the light-scatter distributions. With increasing stimulation, this cluster migrated as a single population from right to left in the histograms. Thus, a partial stimulus provided by a receptor-mimetic ligand induces partial responses throughout the cell population.

The propensity to undergo partial degranulation when intact cells are stimulated by compound 48/80 appears to be related not to the integrity of the plasma membrane, but to the nature of the stimulus itself. This entails the characteristic sequence of events involving activation of phospholipase C (7) and later events consequent to production of inositol phosphates and diacylglycerol. Partial responses were not detected when intact mast cells were treated with different concentrations of the Ca^{2+}-ionophore ionomycin. Fig. 8 shows scatter data from cell suspensions that gave 7, 47, and 72% release. The profiles once again reveal two separate populations that resemble those observed in experiments with permeabilized cells, indicating that direct elevation of intracellular Ca^{2+} also generates an all-or-none response in individual cells.

Discussion

Degranulation of individual mast cells induced by introduction of intracellular effectors into permeabilized cells or by application of the Ca^{2+}-ionophore ionomycin to intact cells occurs in an all-or-none fashion. In contrast, the polycationic agonist compound 48/80 evokes graded responses from individual intact cells.

A possible explanation for the all-or-none behavior of the permeabilized cells is that at a late stage of the secretory pathway, there exists a conditional requirement for exocytosis to proceed. This could be determined by the level of binding of the Ca^{2+} and guanine nucleotide to their respective binding proteins, so that when this threshold condition is satisfied, exocytosis necessarily proceeds to completion. If at any time this condition ceases to be met, secretion must stop. Alternatively, the threshold condition may be determined by irreversible events (e.g., enzyme-catalyzed covalent modifications) leading to the generation of an activated state downstream from the binding of Ca^{2+} and guanine nucleotide. Either way, for secretion from a population of permeabilized cells to proceed to completion, it is known that the conditions for stimulation must be maintained throughout. Depletion of Ca^{2+} (addition of excess EGTA [13]) or GTP (by activation of GTPase on addition of Mg^{2+} to cells initially stimulated by GTP, but not GTP-γ-S [20, 21]) arrests ongoing secretion abruptly. To determine whether this is due to a block at the level of initiation or to the inhibition of ongoing exocytosis, it will be necessary to undertake similar manipulations on single cells.

In addition to providing a means of gaining access to the cytosol, plasma membrane permeabilization by SL-O also allows the leakage from the cell of all solutes including proteins (e.g., lactate dehydrogenase, M_r = 140,000) (14). Exchange of small molecules through the toxin-induced lesions occurs within seconds (22), whereas proteins leak out over

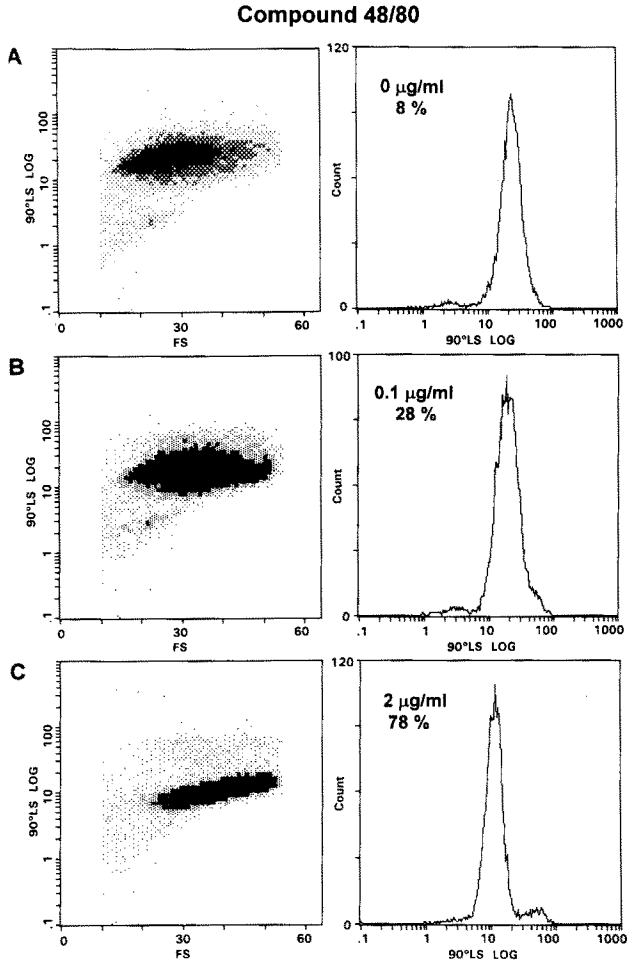

Figure 7. Flow-cytometric analysis of intact mast cells stimulated to secrete by the agonist compound 48/80. The data are presented as in Fig. 4. The concentration of compound 48/80 and the extent of secretion are indicated in each case.

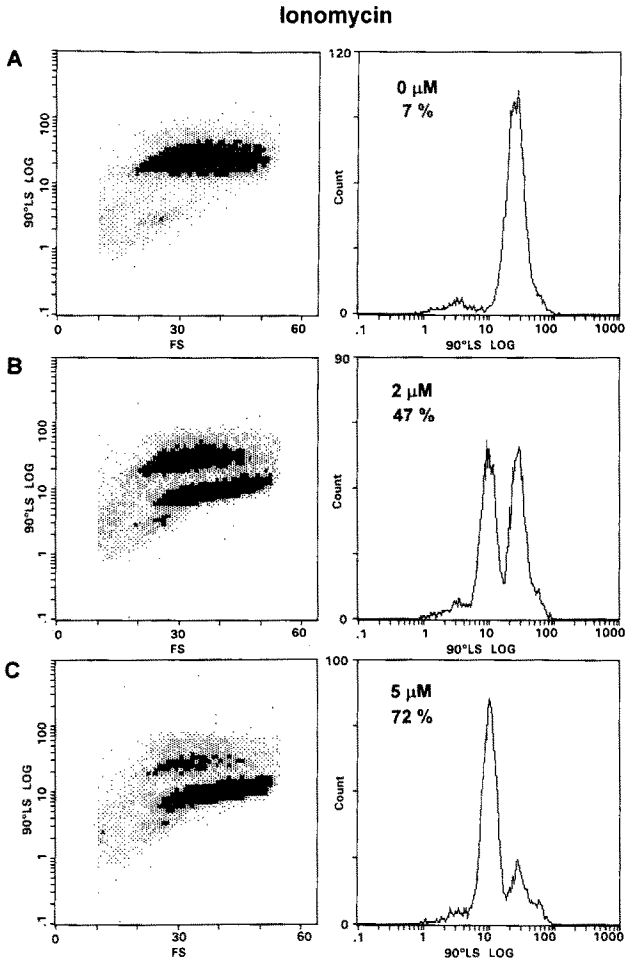

Figure 8. Flow-cytometric analysis of mast cells stimulated to secrete by the ionophore ionomycin. Data are presented as in Fig. 4. The concentration of ionomycin and the extent of secretion are indicated in each case.

a period of 5–20 min depending on size and the nature of their attachment to internal structures. Although some of the factors that leak out over a period of minutes have modulatory roles in the activation of exocytosis (17), it is unlikely that they could account for the all-or-none behavior reported here because intact cells treated with ionomycin also respond in an all-or-none fashion.

The fact that only a proportion of cells respond when a suboptimal combination of effectors is applied may be explained most simply by a variation in the extent of effector binding from cell to cell. This could be due to variations in the local concentrations of the effectors, but we regard this as most unlikely, especially for Ca^{2+}, because in our experiments it was buffered with a high concentration (3 mM) of EGTA. Alternatively, variations in responsiveness of individual cells could be due to a spectrum of sensitivity (effective affinities) of their respective binding proteins. This in turn could be due to variation in the balance of phosphorylation/dephosphorylation states in individual cells because phosphorylations catalyzed by protein kinase C reduce the requirements (i.e., enhance the sensitivity) for Ca^{2+} and GTP-γ-S in SL-O–permeabilized mast cells (15). In this context, it is relevant that both compound 48/80 (7) and antigens that bind and cross-link IgE (2, 9) activate phospholipase C and subsequently protein kinase C.

A consequence of the all-or-none manner of exocytosis is that it becomes legitimate to normalize the progress curves for secretion from permeabilized cells against a common maximum (i.e., 100%). This is shown in Fig. 9, which is based on the data of Fig. 1. Only those cells in the population that recognize the stimulus are represented, and for these, exocytosis goes to completion. When considered in this light, it can be seen that the rate of secretion from the responding cells is rather unaffected by the strength of the stimulus. In this particular experiment, elevation of Ca^{2+} in the range pCa 6.617 to pCa 5 (0.215–10 μM) increased the rate of release less than threefold. A reexamination of previously reported experiments (11, 31) according to this procedure certainly supports this conclusion. In contrast, the delays preceding the onset of secretion are determined (in an inverse manner) by the strength of the stimulus. An analogous result demonstrating that Ca^{2+} primarily regulates the extent, but not the rate, of exocytosis has also been obtained from measurements of catecholamine secretion from electropermeabilized adrenal chromaffin cells (16).

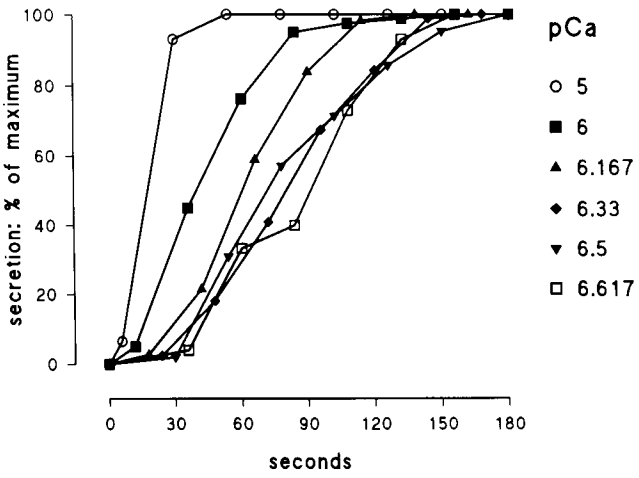

Figure 9. Progress of secretion in response to GTP-γ-S and a range of Ca^{2+} concentrations: normalized data of Fig. 1. For experimental details, see legend to Fig. 1. The progress curves have been redrawn to represent the percentage of maximum secretion achieved over 3 min after stimulation of permeabilized mast cells for a series of graded stimuli.

In intact cells activated by cell surface ligands, the early events of the signal transduction pathway provide a number of opportunities for modulation of the ensuing process. However, the major difference between intact and permeabilized cells lies in the nature of the Ca^{2+} response to activation. In ligand-activated intact mast cells (as in the related RBL-2H3 cells [25] and many other cells responding to receptor-directed agonists [4, 36]), $[Ca^{2+}]_i$ does not simply rise to a new steady level, but fluctuates as a series of transient spikes (Duchen, M. R., and P. E. R. Tatham, unpublished observations). The spatial distribution of these transients within the cytoplasm may also be heterogeneous, particularly when the agonist is applied locally. In the cell population, there will be variability both in the sensitivity of individual cells towards the effectors, as well as variation within individual cells (in time and space) in their concentrations. For this reason, cells exposed to a suboptimal concentration of compound 48/80 appear as a single population in which the extent of degranulation varies continuously (Fig. 7). We have previously shown that single mast cells can undergo successive rounds of degranulation in response to repeated applications of low concentrations of compound 48/80 (35).

Ionomycin transfers Ca^{2+} across membranes by a simple carrier-mediated diffusion mechanism (3), so that all the cells are subject to a similar stimulus. This is unlikely to vary much either between or within the cells. Therefore, unlike compound 48/80, a suboptimal concentration of ionomycin will distinguish between populations of cells that have differing thresholds to stimulation by intracellular Ca^{2+} (Fig. 8). It has previously been shown that although the gross extent of secretion from a population of mast cells varies with the ionophore concentration, $t_{1/2}$ for the secretory reaction remains constant at ~0.6 min (3). This behavior is in accord with the observations described here, suggesting once again that the strength of the stimulus selects the cells, which then proceed to a full degranulation. Once stimulated to secrete, the kinetics of secretion are unrelated to the strength of the stimulus.

GTP-γ-S probably delivers an all-or-none stimulus regardless of the manner of delivery. Exocytosis in individual rat and mouse mast cells has also been studied by recording changes in cell membrane capacitance corresponding to the increase in area that occurs when granules fuse with the plasma membrane (23). This uses the patch-clamp technique in the whole-cell mode so that the cells are effectively permeabilized; after introduction of GTP-γ-S (even under conditions of very low Ca^{2+}), only complete exocytosis is ever observed (26, 28). Conversely, the microinjection technique allows no leakage of intracellular proteins, but again, introduction of any activating concentration of GTP-γ-S evokes the general morphology of extensive degranulation (34) which is at least suggestive of all-or-none responsiveness.

Rat peritoneal mast cells provide an excellent experimental system for studying exocytotic control mechanisms. They are: (*a*) tolerant to permeabilization (secretion is simply measured as release of hexosaminidase [12]); (*b*) offer the possibility of measuring exocytosis or detecting secretion from single cells (8, 35); (*c*) undergo an easily recognizable morphological change during secretion (degranulation); and (*d*) have the propensity to release up to 100% of their stored secretory materials when appropriately stimulated.

These last two attributes have been of particular importance in the present work, in which we have attempted to relate the responses of cell populations to those of single cells. Unlike most other secretory cells, which have to be at the ready, able to respond to stimulation repeatedly (e.g., after meals), mast cells can be regarded as solitary outposts of the immune system. They can remain quiescent indefinitely (30). Moreover, since they act not en masse (as glands) but as single cells, they have to be able to release extensively in order to have any discernible effect on the environment. This has been demonstrated in vivo in both acute and chronic situations, such as following injection of polylysine (29) and in conditions of chronic graft-versus-host disease (6). It is this faculty of mast cells to release 100% of their secretory granules that has provided the means of scaling degranulation at the single-cell level and relating this to the extent of secretion of the marker enzyme hexosaminidase. Such scaling of exocytosis is clearly impractical, or more generally impossible for most other cells, but our observations raise the likelihood that a regulatory mechanism of exocytosis involving a threshold sensitivity to intracellular effectors may be shared by other secretory systems.

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