

Compound versus Multigranular Exocytosis in Peritoneal Mast Cells

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ABSTRACT We have used the whole-cell patch-pipette technique to measure the step increases in the cell membrane capacitance (equivalent to the membrane area) caused by the fusion of secretory granules in degranulating murine mast cells. We have observed that up to 30% of the total membrane expansion caused by degranulation results from large fusion events that cannot be explained by the fusion of single secretory granules. These large events are observed mainly in the initial phase of a degranulation. We have developed a simple mathematical model for a mast cell to test whether these large events are caused by a stimulus-induced, granule-to-granule fusion that occurs before their exocytosis (multigranular exocytosis). Our results suggest that the large fusion events are caused by the exocytosis of granule aggregates that existed before stimulation and that are located at the cell's periphery. We propose a novel mechanism by which granule aggregates can be formed at the periphery of the cell. This mechanism relies on the ability of a transiently fused granule ("flicker") to fuse with more internally located granules in a sequential manner. This pattern may result in the formation of larger peripheral granules that later on can fuse with the membrane. The formation of peripheral granule aggregates may potentiate a subsequent secretory response.

INTRODUCTION

It is well known that the release of mast cell mediators occurs upon the fusion of the secretory granule membrane with the plasma membrane. This fusion results in a net increase in cell surface membrane area and allows continuity between the extracellular space and the granule interior. As exocytosis proceeds, the fusion of subsequent granules is thought to occur with the membrane of previously fused granules rather than with the plasma membrane itself. This pattern of exocytosis has been named compound exocytosis, and allows for the extrusion of secretory products from granules, located deep in the cytoplasm, through intricate tubular pathways formed by the membrane of fused granules (Rohlich et al., 1971). This mode of exocytosis has also been observed in neutrophils (Chandler et al., 1983).

Although it has been suggested from electron microscope studies that the fusion of granules only occurs with the plasma membrane or membrane of previously

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fused granules (Rohlich et al., 1971), it is unresolved whether, upon stimulation, granules can fuse among themselves before fusing with the plasma membrane. This last mode of exocytosis (multigranular) has been reported in adenohypophyseal cells triggered with the calcium ionophore A23187 (Fujita et al., 1983) and in basophils (Dvorak et al., 1981). The mode of exocytosis may determine the size of a secretory response. A compound mode would release single granules, while a multigranular mode of exocytosis will tend to form larger granule aggregates which would then be released as a single package.

Exocytosis has mainly been studied with the electron microscope. With this technique, it is difficult to reconstruct the true size of the granules because thin sections cut randomly through them. Morphometric techniques can reconstruct only nearly spherical granules but granule aggregates may be missed by the plane of the section.

Time-resolved capacitance measurements can follow the step increases in surface area that result from the fusion of secretory granules with the plasma membrane (Neher and Marty, 1982). The size of the steps are an accurate measure of the size of the granule or granule aggregate. Fernandez et al. (1984) noted a population of large capacitance steps (>60 fF) in rat peritoneal mast cells that could not be explained by the dimensions of single secretory granules. These events were attributed to fusion with the plasma membrane of multigranular aggregates that formed upon stimulation with GTP- γ S. Understanding the nature of these large capacitance steps is the purpose of this work.

In this article we extend these measurements to mouse peritoneal mast cells and discuss evidence suggesting that exocytosis in mast cells occurs predominantly in the compound mode and that large fusion events correspond to large granules or granule aggregates that existed before the stimulation of the cell. We also discuss possible mechanisms for their formation.

METHODS

Mast Cell Preparation and Recording Conditions

Mast cells were obtained by peritoneal lavage of mice with a saline solution of the following composition (in millimolar): 140 NaCl, 9 HEPES, 2 CaCl₂, 1 MgCl₂, 6 glucose, 45 NaHCO₃, 0.4 phosphate buffer at pH 7.2. Patch-clamp experiments were done at room temperature (22–24°C) in a similar saline solution, without NaHCO₃ (adjusted to 310 mmol/kg and pH 7.2–7.3). Patch pipette solutions contained (in millimolar): 140 K-glutamate, 10 HEPES, 7 MgCl₂, 0.4 EGTA, 0.2 ATP, 0.1 GTP- γ S, and 1 phosphocreatine (290 mmol/kg, pH 7.2–7.3).

Capacitance and conductance measurements were done using the whole-cell configuration of the patch-clamp technique, and a software-based phase detector (Joshi and Fernandez, 1988) written in "C" for a computer (PC-AT clone). The phase detector operated with a 56 mV peak to peak (830 Hz) sinusoidal command voltage applied to the cell via a patch-clamp amplifier (EPC-7; List Electronics, Darmstadt, FRG). Holding potential was 0 mV.

Size Correction of the Capacitance Steps

At a constant frequency (830 Hz in our experiments) the capacitive current (proportional to the membrane area) gets progressively smaller and deviates significantly from the initial phase

angle as the cell capacitance (area) increases. Therefore, the calibration of the capacitance trace obtained at the beginning of the degranulation cannot be used throughout the entire degranulation. A correcting factor can be found by applying the error equation described by Joshi and Fernandez (1988). This method of correction assumes that only the cell membrane capacitance is changing. Consequently, only cells whose seals remained tight and whose access resistance R_A remained relatively constant, were used for analysis.

Computer Simulation of Mast Cell Exocytosis

Mast cells were simulated by a three-dimensional matrix in which every element of the matrix $A_{i,j,k}$ corresponds to plasma membrane or to a specific granule surface area. The subscripts i , j , and k refer to the location of a particular granule within the matrix, which varies from 0 to N , where N is the number of layers of granules. Each element of the matrix has two associated numbers. (a) A fusion flag $F_{i,j,k}$ that can have a value of 0 or 1. $F_{i,j,k} = 1$ indicates plasma membrane. $F_{i,j,k} = 0$ indicates unfused granule membrane. (b) Size ($S_{i,j,k}$) that indicates the amount of granule, or plasma membrane associated with each element.

At any given time, the total amount of plasma membrane area is then:

$$A(t) = \sum_i \sum_j \sum_k F_{i,j,k}(t) \cdot S_{i,j,k} \quad (1)$$

The outermost elements of the matrix, $S_{0,j,k}$, $S_{i,0,k}$, and $S_{i,j,0}$, all had a fusion flag value of 1 and correspond to the initial plasma membrane. The remaining elements of the matrix had a fusion flag value of 0 and correspond to the unfused granules located in the cell. The values of these elements ($S_{i,j,k}$) were chosen such that they conformed to a Gaussian distribution with characteristics identical to those described for secretory granule diameters in mast cells (Helander and Bloom, 1974). The assignment of these values to any particular element was random.

A degranulation was simulated by assigning a fusion probability, P_{01} , between an element with $F = 0$ and an element with $F = 1$ (plasma membrane), and P_{00} between elements with $F = 0$ themselves. Each element was then polled. If the result was a fusion then the fusion flag of that element was set to 1 only if the fusion was with a plasma membrane element. If a fusion between elements occurred (granule-to-granule fusion) then they both behaved as a single element with a size value equal to their sum. In this last case their fusion flags were 0 until, in a subsequent poll, a fusion between any of the aggregated elements and plasma membrane elements occurred. Then all of the fusion flags of the aggregate change to 1 simultaneously.

Secretory modes are simply set by determining the value of P_{01} and P_{00} . In the compound mode of exocytosis $P_{00} = 0$, and P_{01} has a value between 0 and 1 that is the same for all elements of the matrix. In this case granules can only fuse with plasma membrane or with granules that have already fused with the plasma membrane (any element with $F = 1$). The multigranular mode is obtained by setting $P_{01} = P_{00} \neq 0$, then elements can fuse with each other without contributing to the total area of membrane.

To make the morphological distribution of elements within the matrix more realistic (spherical) the vertices of the cube formed by elements that corresponded to granules were transformed to 1 (plasma membrane). Usually, a cubic matrix of $6 \cdot 6 \cdot 6$ elements was used (117 elements were granules, 99 elements were plasma membrane).

RESULTS

The Size Distribution of Capacitance Steps

During exocytosis, the fusion of a secretory granule with the plasma membrane produces an increase in the surface membrane area by an amount equal to that of the

granule membrane. By monitoring the cell membrane capacitance (Neher and Marty, 1982; Fernandez et al., 1984; Joshi and Fernandez, 1988), which is proportional to the membrane area, we can quantify the surface membrane area of secretory granules once they have fused with the plasma membrane. This particular way of measuring granule sizes has the advantage that regardless of the shape of the granule, the granule membrane is accurately estimated.

Our experiments were done using mast cells from young mice (2–3 wk old) where the cell membrane capacitance typically increased in a stepwise fashion by an average value of two and a half times its resting value. An example of a degranulation is shown in Fig. 1. In this experiment the initial cell membrane capacitance was 2.4 pF, which increased to 6.68 pF at the end of the granulation. As can be observed in the figure, individual fusion events were resolved throughout the entire degranulation.

Histograms of capacitance step sizes were constructed for individual cells. In Fig. 2, a histogram containing 816 steps from nine completely degranulated mast cells is illustrated. As shown by the histogram, most of the step sizes are concentrated in a

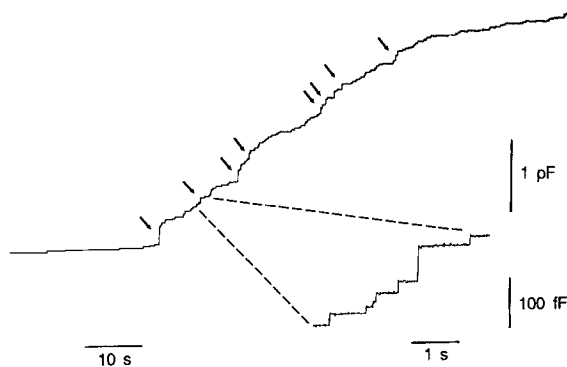


FIGURE 1. Step increase in the membrane capacitance of a mast cell corresponding to the fusion of individual secretory granules with the plasma membrane. An expansion of a small part of the capacitance record is shown in the inset. The arrows point to steps >60 fF.

range between 5 and 60 fF. However, there is a significant population of capacitance steps in excess of 60 fF, similar to that seen in rat mast cells (Fernandez et al., 1984). We have been able to resolve ~ 90 capacitance steps per degranulation (94 in Fig. 1) in the cells included in Fig. 2. The total number of actual secretory granules contained by a cell may be higher because $\sim 10\%$ of the observed capacitance steps could not be measured accurately and were not considered.

A detailed description of the structure and size of mast cell granules is available for rat (Helander and Bloom, 1974) and murine mast cells (Hammel et al., 1983, 1985). These data, obtained from thin sections of unstimulated mast cells, assume that secretory granules have spherical geometry and predict that the fusion of individual granules with the plasma membrane should cause step increases in capacitance ranging between 5 and 60 fF, in contrast to our observations.

The solid line in Fig. 2 shows the amplitude distribution predicted by the diameter frequency distribution obtained from thin sections of rat peritoneal mast cells (Helander and Bloom, 1974). The procedures used to obtain this prediction were similar to those used by Fernandez et al. (1984). As in rat peritoneal mast cells, the

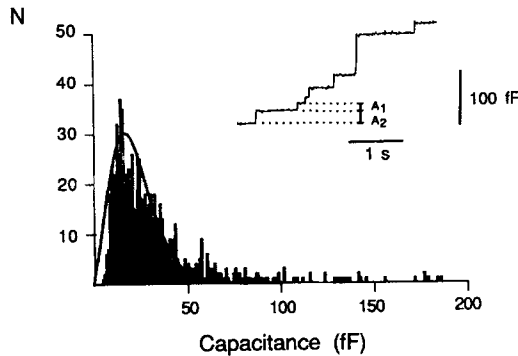


FIGURE 2. Capacitance step amplitude histogram obtained from nine completely degranulated mast cells ($n = 816$). The solid line shows the expected values of capacitance step sizes predicted by electron microscopic studies. The inset shows how capacitance steps sizes are measured. Notice a population of step sizes that significantly exceed the predicted values.

histogram of Fig. 2 shows a significant number of events above 60 fF. These events, although comparatively small in number (83 out of 816), have a significant impact in that they contribute about 30% of the total cell membrane area.

One of the possible explanations for these large (> 60 fF) step increases in capacitance is that, due to the low time resolution of our measurements (one capacitance measurement every 35 ms), a single capacitance step could be made up of two or more simultaneous granule fusions at different plasma membrane sites. To examine this possibility we calculated the probability of observing overlapping fusion events from our records. This can be done by computing the fusion probability distribution function. A histogram showing the probability distribution function for the events observed in one cell is illustrated in Fig. 3. The histogram was constructed by measuring the latency times between consecutive steps ($\Delta t_1, \Delta t_2$, etc. in the inset in Fig. 3). A single exponential was fitted to the data (Fig. 3, *solid line*), with a time constant that averaged 2 s. The probability distribution function, $P(t)$, shown in Fig. 3 gives the probability of finding events separated by a time equal to or longer than t . Then, the probability of finding two events separated by a time smaller than or equal to t , is simply given by:

$$1 - P(t) = (1 - e^{-t/\tau}) \tag{2}$$

Using the measured values of τ , we calculated the probability of observing two

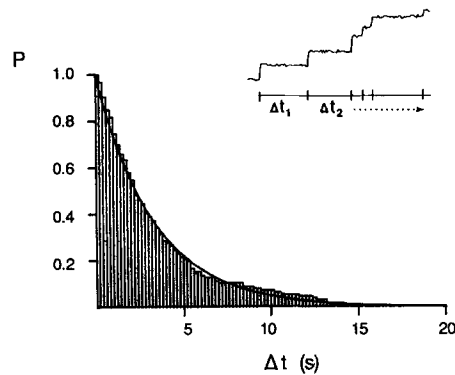


FIGURE 3. Distribution of latency times between successive capacitance steps. The data were obtained by measuring the interval between steps as indicated in the inset. The resulting probability distribution function was fitted by a single exponential.

fusion events with a single measurement period as $1 - P(35 \text{ ms}) = 0.013$. Likewise, the probability that three granules fuse with the membrane within 35 ms and that we measure only one capacitance step is $[1 - P(35)]^2 = 0.00017$; and so forth. This means that out of 100 step increases in capacitance only one event is likely to be due to the fusion of two granules simultaneously, or rather, within 35 ms of each other, and none are likely to be formed by three simultaneous fusions. It is unlikely that our limited time resolution could account for the extremely large step increases of capacitance that we observed in our experiments, as it would require the simultaneous fusion of many granules. We cannot rule out, however, that an unmeasurably fast process is responsible for aggregating granules in a multigranular fashion.

Simulation of Mast Cell Exocytosis

To investigate the effect of the mode of exocytosis on the size of the step increases of capacitance observed during exocytosis, we have created a computer model of a

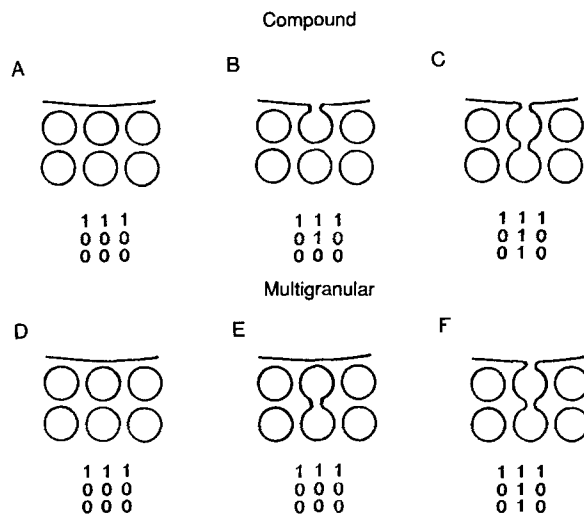


FIGURE 4. Schematic representation of the simulated compound and multigranular modes of exocytosis. The upper part of each panel represents two rows of secretory granules that are close to the plasma membrane. Underneath each one is shown how the granules and the plasma membrane are represented in a matrix form; A, B, and C show the representation of the consecutive fusion of two granules in the compound mode; D, E, and F show the equivalent process as it may occur in the multigranular mode.

mast cell undergoing exocytosis. The model represents the three-dimensional distribution of granules by a cubic matrix. Each matrix element represents either a secretory granule or a patch of plasma membrane (see Methods). Fig. 4 shows how the modes of exocytosis are simulated. Each panel shows a schematic representation of six secretory granules and a patch of plasma membrane. Accompanying these images we show the equivalent sections of their representation as a matrix. For simplicity we show the value of the fusion flag only. Fig. 4, A–C represents a possible sequence of events during compound exocytosis. This mode is simulated by only allowing fusion with elements that already have a fusion flag value of 1. In contrast, Fig. 4, D–F shows a sequence of events during multigranular exocytosis. In this case, fusion among elements is allowed regardless of their fusion flag status. If several elements are fused together but their fusion flags remain at 0 (i.e., Fig. 4 E), they will not contribute to the total surface membrane area until one of these ele-

ments connects with another element with a fusion flag of 1, indicating continuity with the plasma membrane.

To simulate a degranulation we initialized the values of the matrix elements that represent the surface area of the secretory granules, $S_{i,j,k}$ (see Methods). We simulated a compound mode by setting $P_{01} = 0.01$ and $P_{00} = 0$. We also simulated a

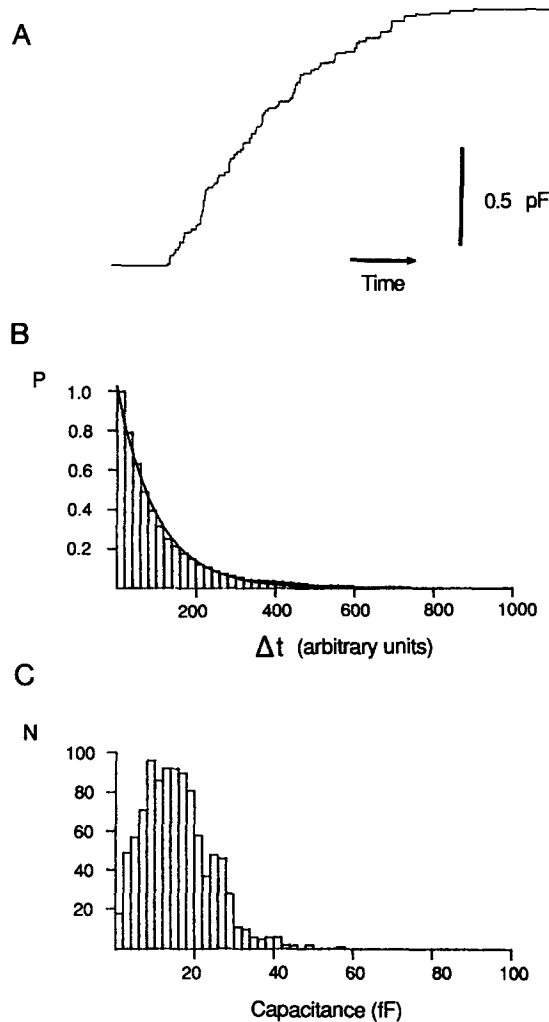


FIGURE 5. Simulation of compound exocytosis. (A) The predicted time course of the capacitance increase during a degranulation. (B) The probability distribution function of latency times between successive steps can be fitted by a single exponential. (C) The capacitance step amplitude histogram shows no steps over 60 fF since granules cannot aggregate before fusing with the plasma membrane.

multigranular mode by setting $P_{01} = P_{00} = 0.01$. In all the simulations the fusion probabilities were kept small so that several polls of the entire matrix were necessary before observing a fusion event. This was important in order to have the "time resolution" necessary for observing all individual events.

Simulation of the two modes reproduced closely the overall time course of the increase in membrane area observed during mast cell exocytosis. Figs. 5 A and 6 A

show the time course of the simulated surface membrane capacitance during a compound and a multigranular degranulation, respectively. The differences in time course are not significant because the time scale is in arbitrary units and can be changed so that both time courses appear similar. Figs. 5 *B* and 6 *B* show the latency distribution that result from a compound and a multigranular degranulation respectively. Again, no apparent difference can be observed. However, the capaci-

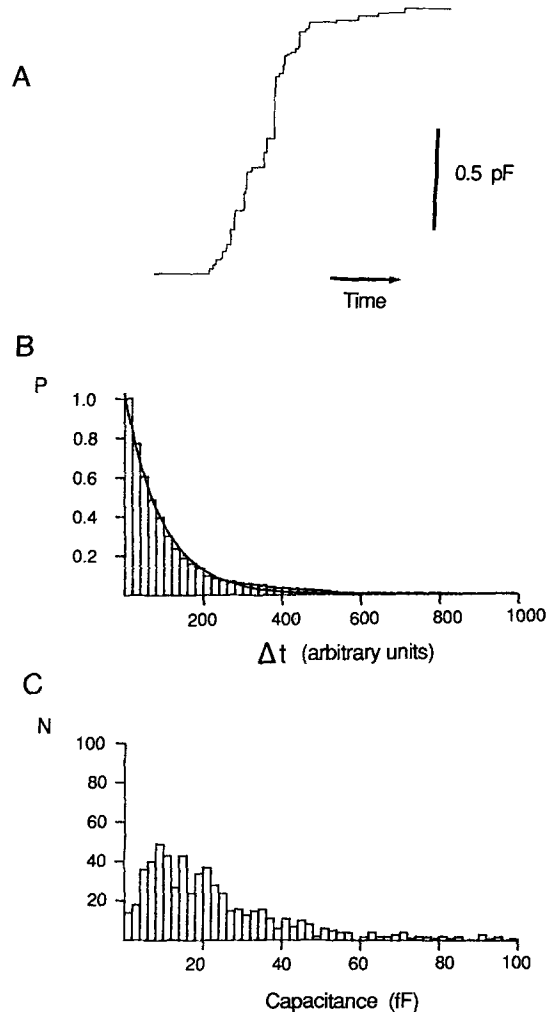


FIGURE 6. Simulation of multigranular exocytosis. (A) The expected time course of the capacitance increase during a degranulation. In this case, the granules have the same probability of fusing with the plasma membrane or among themselves. (B) The probability distribution function can also be fitted by a single exponential. (C) The capacitance amplitude histogram shows a long tail due to steps over 60 fF.

tance step amplitude histograms for both stimulations (Figs. 5 *C* and 6 *C*) were strikingly different. In the compound exocytosis, since no aggregates of granules are formed inside the cell, all capacitance steps observed are due to the fusion of individual secretory granules. Therefore, the histograms show all events to be within the range 0–60 fF (Fig. 5 *C*). On the other hand, the amplitude histogram for the multigranular mode was quite similar to that obtained for real data (Fig. 6 *C*), with a

substantial number of large events (> 60 fF) that correspond to the fusion of granule aggregates with the plasma membrane.

Despite the similar appearance of the amplitude histograms between the real data and the simulated multigranular exocytosis, the time of appearance of the large capacitance steps was quite different (Fig. 7). The time of appearance of large steps was obtained by dividing the entire capacitance record of a degranulation into three intervals; each interval containing the same total increase in capacitance (Fig. 7, *inset*). The percentage of the capacitance contributed by steps over 60 fF to the total capacitance, per interval, was plotted for the cell data (Fig. 7, *open circles*) and for the simulated multigranular exocytosis (Fig. 7, *open squares*).

The cell data (816 steps from nine cells) showed a constant pattern: the largest steps were always observed at the beginning of the degranulation. They were also observed in the middle and less frequently at the end of the degranulation. In con-

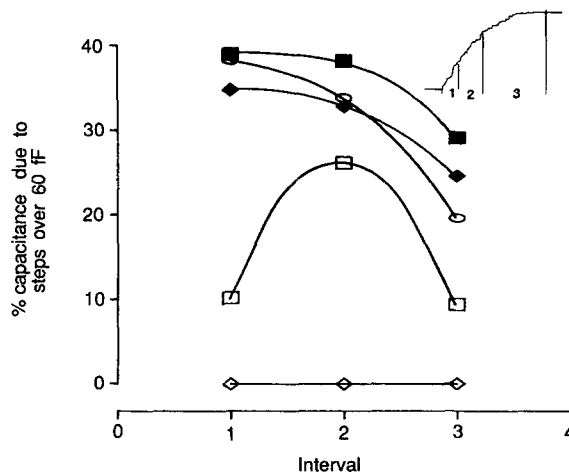


FIGURE 7. Time of appearance of large capacitance steps (over 60 fF) through a degranulation. Every degranulation was divided into three intervals, each containing the same increase in capacitance (*inset*). In the cell data, the events over 60 fF appeared mainly at the beginning and in the middle of the degranulation (*open circles*). In the simulated multigranular exocytosis, large steps are more frequent in the middle of the degranulation (*open squares*). The simulated compound exocytosis shows no steps over 60 fF in any interval (*open diamonds*).

If a set of preexisting granules is created in the cell's periphery, then a simulated compound exocytosis closely reproduces the cell data (*filled diamonds*). A simulated multigranular mode can also approximate the cell data if the probability of granule-to-granule fusion is greatly reduced (*filled squares*).

trast, the simulated multigranular exocytosis showed the largest steps in the middle and never at the beginning of the degranulation. A compound degranulation shows no step increases in capacitance over 60 fF (Fig. 7, *open diamonds*). These results indicate that either a simple compound or a multigranular exocytosis, as simulated by our model, cannot explain the timing of the appearance of large capacitance steps during a mast cell degranulation. This discrepancy is not unexpected. In its simple form, a multigranular exocytosis needs time to build a population of granule aggregates. We therefore expect that as time passes, the contribution made by large multigranular fusion events will go through a maximum as shown in Fig. 7 (*open squares*).

The experimental data of Fig. 7 can be reproduced, qualitatively, if we assume that there was a population of large secretory granules located in the cell's periph-

ery. To simulate this possibility we changed the initial conditions of the matrix elements such that the first shell of granules had value sizes that corresponded to a granule diameter distribution that had a mean value two times larger than that of the remaining granules. With this new set of initial conditions, a simulated compound exocytosis (with $P_{01} = 0.01$ and $P_{00} = 0$) was able to reproduce both the population of large fusion events (steps >60 fF) and the timing of their appearance (Fig. 7, *filled diamonds*). With identical initial conditions, a multigranular degranulation (Fig. 7, *filled squares*) could reproduce the experimental data only if the probability of granule-to-granule fusion, P_{00} , was sharply reduced, in effect approaching a simple compound exocytosis, and allowing the formation of few multigranular structures as a consequence of stimulation. Larger values of P_{00} would invariably produce a maximum, in the middle interval, of the contribution made by capacitance steps over 60 fF (not shown), which is in disagreement with the experimental data.

Formation of Granule Aggregates in the Cell's Periphery

The results of Fig. 7 suggest that there is a population of large granules or granule aggregates near the periphery of a resting mast cell. The recordings of Fig. 8 may

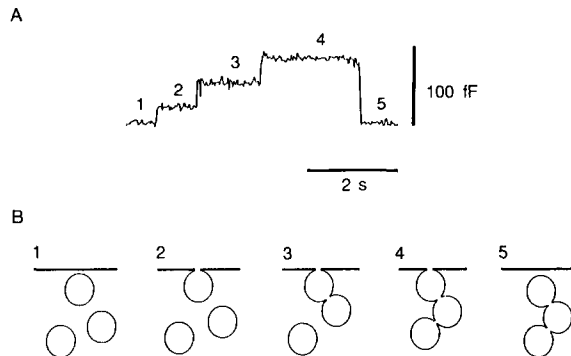


FIGURE 8. Possible mechanism by which secretory granules can form aggregates of granules through "flicker." (A) Three different granules of 22, 30, and 32 fF fusing in succession with the membrane. After 2 s a backstep of 84 fF can be observed. (B) A possible sequence of events that may explain this observation.

provide an explanation for how these granule aggregates can be formed. Fig. 8 shows the consecutive fusion of three distinct secretory granules increasing the cell membrane capacitance by ~ 22 , 30, and 32 fF, respectively. Surprisingly, shortly after this increase, a step decrease in capacitance of 84 fF was observed. Note that the step decrease in capacitance matches, exactly, the sum of the three fusion events. Sequences of two or three fusions followed by a single matching step capacitance decrease have been observed at the beginning of some, but not all, degranulations. A likely explanation for this observation is shown in the sequence of Fig. 8 B. This interpretation is based on the ability of secretory granules to form transient connections with the plasma membrane, a phenomenon called "flicker" (Fernandez et al., 1984; Breckenridge and Almers, 1987a, b; Zimmerberg et al., 1987; Alvarez de Toledo and Fernandez, 1988). A peripheral granule "flickers" and undergoes a transient fusion with the plasma membrane. While it is fused, the granule acquires the ability to fuse with more internally located granules in a sequential form, so more granules can fuse in a chain, as it is usually described for the com-

pound mode. If, at this point, the peripheral granule ends its "flicker," and is no longer connected with the plasma membrane, a larger structure will perhaps remain, composed of three aggregated granules.

DISCUSSION

Electron micrographs have shown that exocytosis may proceed in different modes depending on the cell types considered and the stimulus that triggered exocytosis. Compound exocytosis seems to be the major mechanism of secretion in mast cells stimulated either with compound 48/80 (Rohlich et al., 1971), bee venom (Bloom and Haegermark, 1967), or with antigens in sensitized cells (Anderson et al., 1973). Also, compound exocytosis seems to occur in human platelets (Morgenstern et al., 1987) and neutrophils (Chandler et al., 1983; Chandler and Kazilek, 1986). On the other hand, multigranular exocytosis may account for secretion in anterior pituitary cells triggered with the Ca^{++} ionophore A23187 (Fujita et al., 1983) or phospholipase A_2 activators such as mastoparan and melittin (Kurihara et al., 1986), and in basophils (Dvorak et al., 1981). In basophils the secretory granules seem to form large aggregates inside the cell ("degranulation sacs") (Dvorak et al., 1981) that finally connect to the cell exterior through narrow connections with the plasma membrane.

These differences in pattern of granule fusion between different cell types and secretory stimulus may indicate that several intracellular messengers can be activated, leading to distinct patterns of exocytosis, or they may also be an indication of the inherent difficulties of the electron microscope in unambiguously assessing whether granules have fused or if they are still separate units.

Capacitance measurements of the cell membrane cannot substitute for the ability of the electron microscope to image the secretory granules in a resting unstimulated cell. However, they can accurately measure the contribution to surface membrane area that a granule or granule aggregate makes when it fuses with the plasma membrane. These measurements are able to detect even very small pores (Fernandez et al., 1984; Breckenridge and Almers, 1987*a, b*; Zimmerberg et al., 1987; Alvarez de Toledo and Fernandez, 1988), that may connect several neighboring granules together. Small fusion pores connecting two or more granules would be hard to detect with the electron microscope as they would surely be missed by the plane of section.

Using the capacitance technique, our experiments have confirmed the presence of a population of large (>60 fF) fusion events in degranulating murine mast cells. There are several plausible explanations for the presence of these large fusion events. Initially we considered that the multigranular mode of exocytosis could explain them, however, the substantial discrepancy in the time of appearance of these large events suggests otherwise (see Results). The fact that these large fusion events occur at the beginning and in the middle of a degranulation indicates that these granules are located close to the plasma membrane. A similar observation has been reported in the human eosinophils (Henderson and Chi, 1985). In this preparation, when treated with a small concentration of the calcium ionophore A23187, there is exocytosis of the granules with the largest diameters, the small granules remaining in the cell interior.

The implication is that the large capacitance steps correspond to the fusion of large individual secretory granules that preexisted stimulation of the cell. A possible explanation for the presence of these granules is that the *trans* Golgi network sometimes produces some very large granules during the initial phases of granulogenesis. Alternatively, it is possible that granule aggregates can be formed through transiently fused granules by a mechanism similar to that described in Fig. 8B (see Results). This pattern of fusion may occur spontaneously before the onset of the stimulus for exocytosis. Granule shapes that clearly indicate the fusion of two or more mature granules have been observed in unstimulated mast cells (Hammel et al., 1983) and mast cells stimulated with polylysine (Padawer, 1970).

In conclusion, we do not need to invoke a multigranular mode of exocytosis to explain large secretory events. We propose a novel mechanism for the formation of large secretory granules in the periphery of the cell: namely, through pore formation a peripheral granule can establish temporary contact with the plasma membrane, and when this occurs, the properties of that membrane are changed in such a manner that other granules can fuse with it forming a larger granule aggregate. These larger granule aggregates would be located at the periphery, and could perhaps be formed under very low levels of stimulation where a small amount of flicker may take place. If this were true, the main effect of the low level stimulation would be to increase the size of the peripheral granules. Larger secretory granules in the periphery of the cell would potentiate a secretory response by releasing a larger dose of mediators in response to a stimulus. This hypothesis can be tested with the methods presented here and by using cultured mast cells exposed to low levels of stimulation.

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