PLASMA MEMBRANE FOLDS ON THE MAST CELL SURFACE AND THEIR RELATIONSHIP TO SECRETORY ACTIVITY

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ABSTRACT

Changes in the surface morphology of secreting mast cells have been followed by scanning electron microscopy. Mast cells isolated from the rat peritoneal cavity have folds of plasma membrane that form snake-like ridges on their surfaces. Fold length varies considerably from cell to cell, whereas fold width and depth appear to remain relatively constant. To assess the possible relationship between secretory activity and surface folding, a semiquantitative method was used for measuring fold length in control and secreting populations. A positive correlation is found between secretion of histamine and the extent of membrane folds on the mast cell surface. The source of the membrane required for fold formation is probably secretory granule membrane incorporated into the plasma membrane as a result of exocytosis. Furthermore, a distinct cell type devoid of surface folds, designated as a raspberry-type cell, is found to occur as an integral part of a normal population of mast cells. This cell type is resistant to stimulation by polymyxin.

KEY WORDS mast cell · histamine secretion · membrane folds · cell surface morphology · scanning electron microscopy

The surface features of a cell are an expression of the cell's response to its internal and external environment. Cell surfaces change as a function of altered physiology, for example, during cell cycle (18), in transformed cells (19), in response to environmental irritants (17), etc. The experiments reported here were undertaken to determine a possible relationship between surface features and secretory activity in the rat peritoneal mast cell.

The mast cell synthesizes and stores histamine, among other secretory products, and undergoes compound exocytosis when stimulated to secrete (6). Mast cells observed in the scanning electron microscope (SEM) have, as characteristic surface features, folds of plasma membrane that form curved ridges in anastomosing patterns (3, 9, 23), corresponding to microvillus-like projections observed in thin sections (11, 20). The folds are also visible on freeze-fracture faces of plasma membrane as cross-fractured areas forming snake-like patterns (4). Previous studies of the mast cell in SEM (9, 23) reported the presence of these folds primarily on unstimulated cells. A cell type, devoid of folds, was also described, designated as the raspberry-type cell, which was dismissed as being an artifact of the preparation (9). When cells were exhaustively stimulated to secrete, they lost their surface folds, developed deep cavities, and partially collapsed, revealing sharp contours of underlying granules. The dosages of secretagogue and incubation times used in these previous studies were many times in excess of the conditions re-

quired to obtain 100% histamine release (12).

In the experiments reported here, intermediate stages of secretion are obtained by using milder conditions of stimulation, i.e., lower dosage of secretagogue, shorter incubation times, and lower temperature. Histamine release is measured to assess the secretory activity of the cells. In this way, changes in the surface of secreting mast cells can be studied relative to the secretory activity of the population. Since the mast cell is essentially nonmitotic, cell cycle is not a factor in influencing its surface features. Therefore, in this case, changes in surface features probably reflect other physiological responses, the most likely of which is secretory activity.

MATERIALS AND METHODS

Cell Collection and Incubation

Mast cells are collected from the peritoneal cavities of exsanguinated male rats, 150–180 g, of the Long-Evans strain (U. C. Berkeley colony) by recovering phosphate-buffered saline (PBS) (pH 7.2) pipetted into the cavities (20). The cell suspensions, initially containing ~5% mast cells, are enriched to >70% mast cells by using discontinuous Ficoll (Pharmacia Fine Chemicals, Piscataway, N. J.) gradients (1). Mast cells derived from three to eight rats are pooled and resuspended in PBS at 18°C. A controlled amount of histamine secretion is obtained by adding polymyxin B sulfate (4 μg/ml of incubation mixture). Samples are taken 60 s after stimulation for assay of histamine release and examination in SEM.

Histamine Assay

Ice-cold PBS is added to each sample at the end of an incubation period to stop secretion. The samples are centrifuged (200 *g*, 4°C, 5 min) and histamine determinations are made on each supernate and pellet pair by the method of Bergendorff and Uvnäs (2). Histamine is completely extracted from each pellet by boiling in 0.1 N HCl for 5 min. Dilutions are made with H_2O. Samples of supernate containing undiluted PBS form precipitates during the assay which do not interfere with determinations if removed by centrifugation or filtration before reading. Histamine release is expressed as the percentage of histamine in the supernate (supernatant histamine plus pellet histamine equals 100%).

Sample Preparation for Scanning Electron Microscopy

Mast cells are fixed in suspension with ice-cold 2% glutaraldehyde in 0.2 M Millonig's buffer (pH 7.4) and kept at room temperature for 1 h, then washed once (Millonig's buffer plus 6% sucrose) and postfixed for 1 h in 1% OsO_4 in Millonig's buffer at 4°C. The cells are kept in suspension throughout alcohol dehydration, transferral to 100% Freon TC (E. I. du Pont de Nemours & Co., Wilmington, Del.), and placement in B.E.E.M. capsule chambers (Better Equipment for Electron Microscopy, Inc., Bronx, N. Y.) with Flotronics silver membrane filters (Selas Corp. of America, Spring House, Pa.) (0.45 μm pore size) covering one end. The cells are critical-point dried in Freon 13 (E. I. du Pont de Nemours & Co.). Flotronics filters give high rates of recovery for cell suspensions (7). Surface artifacts caused by Flotronics filters (25) are avoided by fixation of cells before contact with them (7). Polylysine (14) is not used for attachment of cells to glass cover slips because of the possible introduction of surface artifacts (16).

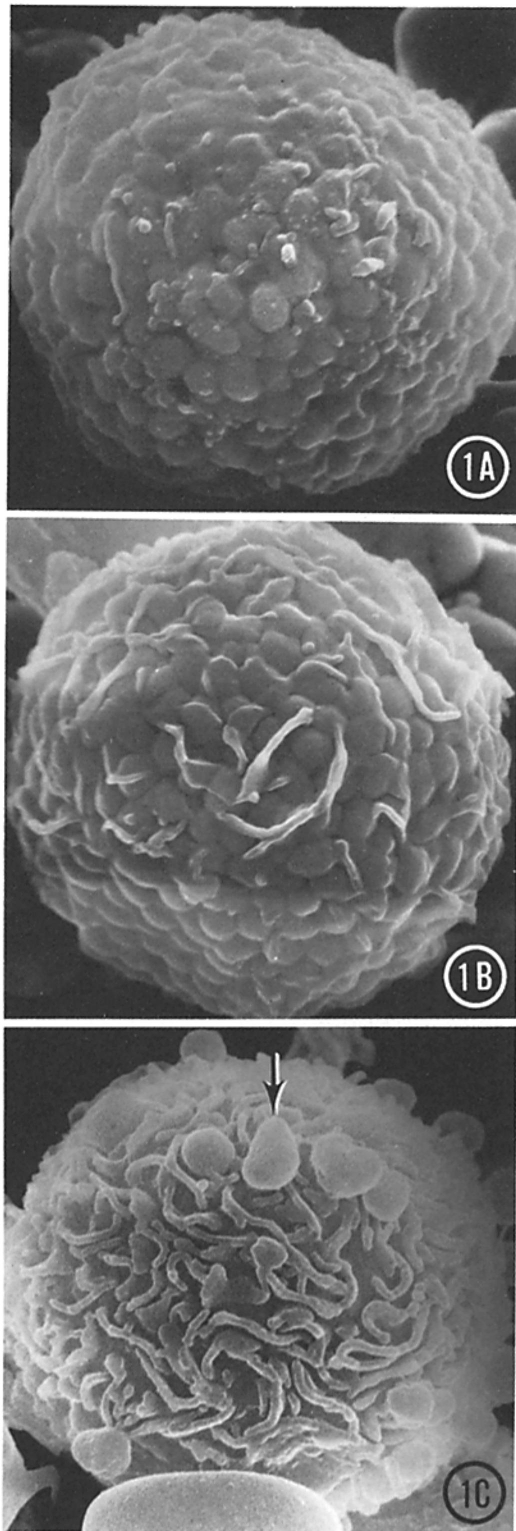
Pieces of filter containing the critical-point dried cells are glued onto specimen stubs with silver paste, coated with gold under vacuum on a tilting-rotating stage, and examined in an ETEC or Coates and Welter CWIC scanning electron microscope. Micrographs are taken with Polaroid type 55 P/N film.

RESULTS

Mast cells observed in SEM have folds of plasma membrane that project from their surfaces. The folds form snake-like ridges in anastomosing patterns (3, 9, 23). In between folds, contours of underlying granules are visible. No microvilli are observed (Fig. 1). Therefore, the surface projections from mast cells which were previously identified as microvilli in thin sections (11, 20) correspond to these folds of plasma membrane viewed in cross section.

The folds appear to be fairly uniform in width on the same cell and from one cell to another, averaging ~0.1 μm. Fold depth, as viewed at the cell edge, varies to some extent, ranging from 0.3 to 1.0 μm with 70–80% of the folds ~0.8 μm deep. Fold length is the dimension which shows the greatest variability from one cell to another. Differences in fold length are extremely apparent from cell to cell, whereas fold width and depth tend to remain fairly constant.

Variability in fold length is the factor which most accounts for the heterogeneous appearance of the mast cell in SEM with respect to surface features. The cells in Fig. 1 are arranged in a sequence of increasingly folded surfaces (increasing fold length). Some cells lack folds entirely (the raspberry-type cell) (Fig. 1*A*) and the contours of underlying secretory granules are visible. Cells with extensively folded surfaces (Fig. 1*C*) often have the extruded contents of several granules adhering to them (arrow), while underlying granule contours are almost totally obscured by folds.



Furthermore, the complete range of cell surface appearances is present in both control and stimulated populations.

The secretion of histamine by mast cells, under the conditions of stimulation used (incubation in PBS at 18°C for 60 s in the presence of 4 μg/ml polymyxin B sulfate), was 64.2 ± 4.0% (n = 3), while the corresponding histamine release by control cells, incubated in the absence of polymyxin B sulfate, was 9.9 ± 1.0% (n = 4).

To determine whether the surface features of mast cells change as a result of stimulation and secretion, a qualitative evaluation of the extent of folding was carried out as follows: Each of two preparations of cells was divided into control and stimulated populations. 50 cells from each population were randomly selected in SEM at low magnification (× 100) at which any surface detail is indistinguishable. Magnification was then increased to × 10,000, and if the cells met the criteria of being undamaged, spherical, and unobstructed, they were photographed. In this manner, 200 cells were systematically photographed.

For each cell, a number was assigned on a scale of 1 to 3, depending on the extent of folds on the cell surface. Category 1 represents cells with very few folds (including raspberry-type cells), category 2, cells with an intermediate range of folds, and category 3, cells with extensively folded surfaces. The three cells in Fig. 1 are representative of each category. The numbers of cells falling into each category were compared in the paired control and stimulated populations (Table I). The results show that more cells have extensively folded surfaces in the stimulated populations than in the control populations.

To confirm the results obtained with the qualitative evaluation, a semiquantitative method was developed for measuring fold length per cell. If the simplifying assumption is made that fold width and depth are relatively uniform from cell to cell, then fold length becomes the variable that allows direct comparison of one mast cell to another. 100 randomly selected cells from a third preparation, divided into control and stimulated populations,

FIGURE 1 The various appearances of the mast cell surface in SEM. These cells are taken from both control and stimulated populations and are arranged in order of increasingly folded surfaces. 1*A*: Category 1: a raspberry-type cell; 1*B*: Category 2: cell with an intermediate range of folds; 1*C*: Category 3: cell with an extensively folded surface. Several extruded granules are seen on the cell surface (arrow). All × 8,750.

were photographed as described above, and micrographs were printed at a final magnification of × 20,000. Fold length measurements were carried out according to the method described in Fig. 2. For each cell, a ratio of fold length in micrometers per square micrometers of surface area was calculated, and the distributions of cells according to these ratios were compared in the paired control and stimulated populations (Fig. 3). Fold length varies over a range from 0 $\mu m/\mu m^2$ surface area (raspberry-type cells) to ~2.4 $\mu m/\mu m^2$ surface area, and the distribution resembles a poisson distribution (Fig. 3*a*). However, the proportion of raspberry-type cells, which varies from 6 to 12%, is higher than would be predicted from a typical poisson distribution. There is an overall shift in distribution as a result of stimulation (Fig. 3*b*), again demonstrating a positive correlation between secretion of histamine and the appearance of membrane folds.

TABLE I
Qualitative Evaluation of Surface Folding Relative to Secretory Activity

Population	Category 1: very few folds	Category 2: intermediate folds	Category 3: extensive folds
		no. of cells	
Control	11	23	16
Stimulated*	13	12	25
Control	11	31	8
Stimulated*	12	23	15

* Stimulated with polymyxin B sulfate, 4 $\mu g/ml$, at 18°C for 60 s.

The average length of folds per cell for the stimulated population increases ~23% as a result of secretion of 64% of the histamine present (Table II). The relationship between the secretory activity of a population and cell size is shown in Table III. The average cell radius decreases ~5% upon stimulation. However, this shrinkage alone cannot account for the above increase in membrane fold length (Fig. 4). At most, 0.07 μm folds/μm^2 surface area can be generated from the membrane area made available by cell shrinkage. Since the average increase in fold length from control to stimulated cell is ~0.3 $\mu m/\mu m^2$ surface area (Table II), the 0.07 μm folds/μm^2 can account for, at most, ~23% of the 0.3 $\mu m/\mu m^2$ membrane needed to generate the folds.

Prolonged stimulation of cells for 15 min (still at 18°C) results in extensive cell damage and lysis. Cell recovery is checked by counting cells in a hemacytometer. After prolonged stimulation, ~27% of the cells lyse and are not even recovered

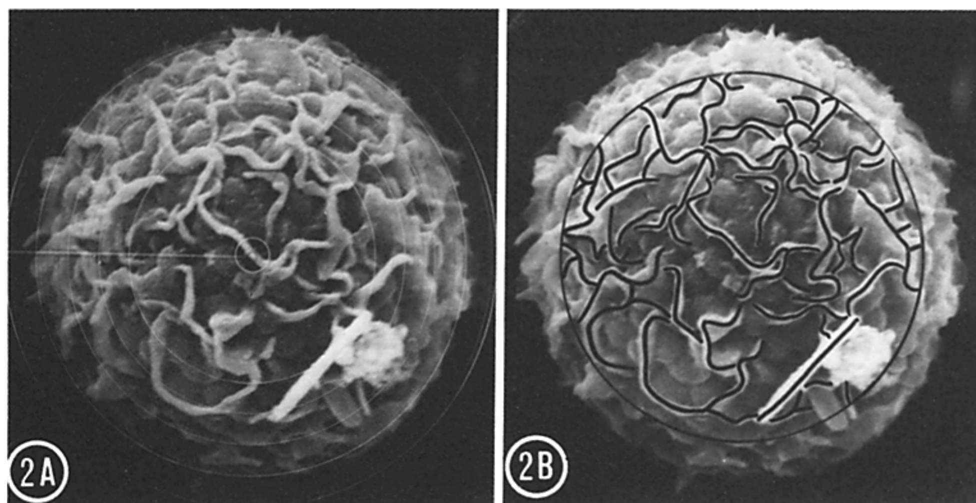
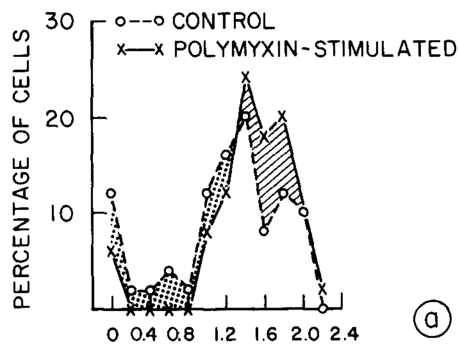


FIGURE 2 Method for measuring fold length on mast cells. 2*A*: A clear plexiglass circle with concentric rings etched on it at 1-cm intervals is superimposed over a scanning micrograph of a mast cell originally printed at × 20,000 and reproduced here at × 8,750. The center point and radius of the cell are determined. 2 *B*: A concentric circle whose radius is 80% of the radius of the cell is drawn on the micrograph. The area falling within this circle corresponds to 20% of the total surface area of a smooth sphere. The folds of membrane lying within this circle are traced with a map reader to obtain a linear measurement. The data for each cell are expressed as a ratio of fold length (μm) to sphere surface area (μm^2). × 8,750.



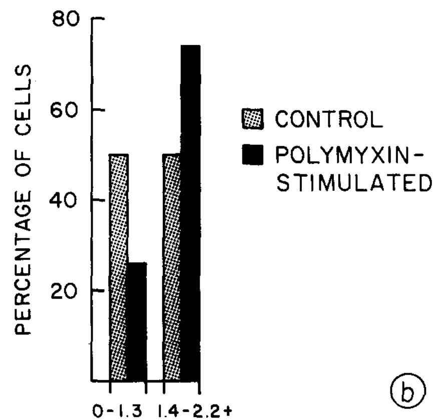


FIGURE 3 The distribution of cells according to their membrane surface folds in paired control and stimulated populations. 3*a*: The cells are arranged in categories of 0.4-μm increments of their ratios of fold length to surface area. The shift in distribution after stimulation is emphasized by the stippled vs. striped areas. 3*b*: The histogram presents the same data as in Fig. 3*a*, reduced to two categories: 0–1.3 and 1.4–2.2+ folds (μm)/μm^2 surface area.

TABLE II
Length of Folds per Surface Area

Population	Average length of folds
	μm/μm²/Cell
Control	1.3 (n = 50)
Stimulated*	1.6 (n = 50)
Percent Increase	23%

* Stimulated with polymyxin B sulfate, 4 μg/ml, at 18°C for 60 s.

for further study. Of the remaining 73%, ~40% are damaged as revealed by examination in SEM, showing collapse, partial lysis, and loss of plasma membrane integrity. In summary, upon prolonged stimulation, 27% of the cells are not even recovered, and 29% are damaged, leaving only 44% of the initial population intact. Of this 44%, a great majority (>80%) appear to be raspberry-type cells or cells with relatively few folds (0–1.0 $\mu m/\mu m^2$, Fig. 3*a*). Therefore, these remaining cells must be relatively resistant to polymyxin treatment, and the raspberry-type cell appears to be particularly insensitive to polymyxin relative to other mast cells.

TABLE III
Mast Cell Size as a Function of Secretory State

Population	Radius	Area of a smooth sphere calculated from radius
	μm	*μm²*
Control	4.34 ± 0.05	237
Stimulated*	4.10 ± 0.05	211
Percent Decrease	5.5%	
Membrane available as a result of radius change		26

n = 50 for both populations. The data are expressed as the means of the n, ± SEM.
* Stimulated with polymyxin B sulfate, 4 μg/ml, at 18°C for 60 s.

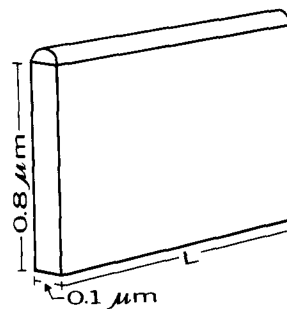


FIGURE 4 Contribution of cell shrinkage, associated with secretion, to increase in membrane fold length. The average width of folds = 0.1 μm, and the average depth of folds = 0.8 μm (both dimensions approximated from scanning micrographs). The length of fold with these dimensions which can be generated from 26 μm^2 membrane area (Table III) is 15 μm;

$$0.8L + 0.8L + 0.1\left(\frac{\pi}{2}\right)L = 26\ \mu m^2$$
$$L = 15\ \mu m.$$

15 μm per 211 μm^2 surface area gives a ratio of 0.07 $\mu m/\mu m^2$.

DISCUSSION

The mast cell, observed in SEM, has folds of plasma membrane that form snake-like ridges on its surface. These folds correspond to the microvillus-like projections previously observed in thin sections. Other cells, observed in SEM as having similar folds, distinct from microvilli, are dissociated epithelial cells (24).

The mast cell folds appear to vary in length from cell to cell to a far greater extent than they vary in width and depth. Some cells lack folds entirely, whereas other cells are completely covered with them. The complete range of cell surface appearances is found in both control and stimulated populations. However, qualitative evaluation of 200 cells in two separate preparations suggested that more cells have extensively folded surfaces in the stimulated populations than in the control populations. To test this observation further, a semiquantitative method was devised to compare the extent of plasma membrane folding on control vs. stimulated cells. The length of folds per cell was chosen as the variable allowing comparison of one mast cell to another. A positive correlation between histamine secretion and fold length was found. This method tends to minimize the difference between control and stimulated cells, because observations in transmission electron microscopy (16) have tentatively suggested that fold depth may actually increase with stimulation.

Expansion of the surface area of the mast cell plasma membrane as a result of exocytosis was first reported by Kinsolving et al. (10), using an increase in acridone-binding capacity as an indicator of newly available membrane surface area. The increase in plasma membrane area was related to histamine secretion (15), with 63% histamine release corresponding to a 350% plasma membrane expansion. The apparent discrepancy between those results and the results presented here is due to the fact that in the experiments of Kinsolving et al., the internal cavities formed by compound exocytosis are included in the measurement. The current observations on plasma membrane expansion are limited to the increase in surface area visible as membrane folds on the cell surface.

Cell shrinkage associated with secretion will not provide sufficient membrane for generation of folds in stimulated cells. The rapidity of the change (within 60 s) probably precludes *de novo* membrane synthesis as the source of the needed membrane. Therefore, the membrane for fold formation probably comes from granule membrane incorporated into plasma membrane after membrane fusion and exocytosis. Incorporation of vesicle membrane into plasma membrane after release of secretory product has been shown to occur in several secretory systems: in frog neuromuscular junction after prolonged stimulation (8), in *Tetrahymena* in which synchronous release has been induced (21, 22), in sperm after acrosome discharge (5), and in the secretory cells of the duck salt gland (13).

The raspberry-type cell, the cell that lacks membrane folds, does not appear to be a damaged cell or an artifact of preparation, in contrast to views previously stated (9, 23). Rather, it is part of the normal mast cell population, and its proportion is higher than would be expected from a poisson distribution. This cell is resistant to damage by prolonged exposure to polymyxin under conditions which damage other mast cells. The raspberry-type cell may be harder to induce to secrete, due to its having less surface area, or having immature membrane which may lack necessary receptor molecules for stimulation. The results suggest that cells with greater surface area (extensively folded surfaces) are more susceptible to stimulation, having perhaps more receptor molecules available. One might speculate that additional receptors may be contributed by the interior surface of the granule membrane which, upon fusion, becomes the exterior surface of the plasma membrane. A positive feedback mechanism may be involved in the explosive nature of mast cell secretion at physiological temperature: stimulation → increase in folds (receptors) → further stimulation. A positive feedback effect could account for previously stimulated cells being especially susceptible to damage and lysis by further stimulation.

Raspberry-type cells may represent different stages of mast cell maturation, during either (*a*) differentiation, or (*b*) recovery after depletion of secretory products. As for the first possibility, a precursor cell of the fully differentiated mast cell still has not been positively identified. With regard to the second possibility, the mast cell should theoretically be able to synthesize and package new product, to "recycle", because its plasma membrane remains intact and all necessary cytoplasmic organelles are present. However, the process of recovery after depletion has not been well studied in mast cells. Finally, the raspberry-

type cell may represent a less mature form of a mast cell that is less susceptible to stimulation.

The fact that raspberry-type cells can be selected for by their ability to survive prolonged stimulation (15 min) may provide a means for isolating them from other cells present. Thus, problems of differentiation, recycling, membrane sites, etc. could possibly be specifically studied in these cells.

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