MEMBRANE FUSION DURING MAST CELL SECRETION

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INTRODUCTION

Much evidence has been adduced to support exocytosis as the mechanism utilized by a variety of cells to secrete substances stored in intracellular vesicles or granules. Since in all instances the vesicle or granule is bounded or surrounded by a membrane which in turn is separated from the external milieu by the plasma membrane, exocytosis must involve an interaction between two membranes leading to the formation of a channel through which the secretory product leaves the cell.

Secretion of histamine and other secretory granule components by mast cells probably occurs not only at the cell surface but also deep in the cell (24). The extent of membrane interaction required during secretion might be expected to make the mast cell an auspicious object for the investigation of membrane interactions. An ultrastructural search for examples of membrane interaction during mast cell secretion yielded the preliminary results reported here.

MATERIALS AND METHODS

Peritoneal cells including mast cells were collected from the peritoneal cavities of 2-4-month old male rats (CD, Charles River Breeding Laboratories, Inc., Wilmington, Mass.) as previously described (24). The cells were washed once in heparin-free balanced salt solution and samples containing 2-5 × 10^6 mast cells/ml by hemacytometer count were distributed in 1.5 ml aliquots in test tubes. Cells were kept at either 30°C or 37°C, and secretion was induced by the addition of polymyxin B sulfate to a final concentration of 0.5-4.0 µg/ml. Inactive and secreting cells were fixed by the direct addition of an equal volume of 4% buffered glutaraldehyde (0.1 M cacodylate buffer, pH 7.4) to a cell suspension. The cells were fixed for 1 or 2 h at room temperature, washed in 0.1 M cacodylate buffer, postfixed with 1% osmium tetroxide in collidine buffer, pH 7.0, for 1 h at 0°C, washed with pH 5.0 HCl solution, and stained with 0.5% uranyl acetate in collidine buffer, pH 7.0. The cells were washed with water and collected in agar (15). The agar pellet was diced, and the bits dehydrated and embedded in Epon812. Thin sections were stained with uranyl acetate and alkaline lead reagent (26) and examined in an AEI-6B electron microscope.

RESULTS

In adequately fixed, resting mast cells, each histamine storage granule is surrounded by a unit membrane (Fig. 1). An approach to closer than 100 Å of the outer leaflets of adjacent perigranule membranes or of a perigranule membrane and
the plasma membrane is rare. When mast cells are examined 5 min after their exposure to 0.5 µg/ml polymyxin B sulfate, the cells are seen to be penetrated by channels of extracellular space (Fig. 2). Many altered granules are present in these channels. Under these conditions, 30-40% of the cell histamine is released (24).

When fixative is added to the cell suspension 5 s after polymyxin B sulfate, the peripheral granules are found to be the most frequently involved in the secretory process (Fig. 3). The membranes originally encompassing some of the peripheral granules just beneath the plasma membrane establish continuity with the plasma membrane, effectively externalizing the granules (Fig. 4). Even at this early time some cells exhibit ex-
tensive involvement of granules including those deep in the cell (Fig. 5). A marked alteration of granule structure is evident in granules that are in contact with extracellular medium. The electron-opaque homogeneous granule (Fig. 1) is progressively transformed to a dispersed fibrous meshwork (Figs. 2-5). Close approach of adjacent perigranule membranes and perigranule and plasma membrane is frequently seen (Fig. 6). However, the membranes of stimulated cells are particularly difficult to preserve and artifacts are frequent (Fig. 7).

Several micrographs of pentalaminar fusion of membranes have been obtained with cells incubated at 30°C (Figs. 8 and 9). All of these involve adjacent perigranule membranes. A few micro-

Figure 2 This mast cell was fixed after 5 min of exposure to polymyxin B sulfate 0.5 µg/ml at 30°C. Altered granules are present in spaces formed by fusion of the membranes around the individual granules. No continuities between the extracellular region and the spaces containing the altered granules are evident. A few granules are present that retain their homogeneously dense appearance. The cytoplasm shows no degenerative changes; mitochondria, microtubules, small vesicles, and the centriole are unaffected by the secretory process. × 15,000.
FIGURE 3 A mast cell fixed by the addition of glutaraldehyde to a suspension of cells 5 s after the addition of 0.5 µg/ml polymyxin B sulfate. At this early time period only a few of the most peripherally situated granules have been extruded. X 4,500.

FIGURE 4 A higher magnification of a granule being discharged at the surface of a mast cell treated as described in Fig. 3. An intact unit membrane is traceable around the altered granule and is continuous with the plasma membrane. X 60,000.

FIGURE 5 A mast cell treated as described in Fig. 3. Involvement of most of the granules is evident at this early time. X 4,500.

graphs are suggestive of an early stage of pore formation involving a perigranule membrane and plasma membrane (Fig. 10), but in none of these are the membrane leaflets distinct enough to be entirely convincing. I have observed neither tri-laminar membranes nor diaphragms lacking unit membrane structure separating two granules or a granule and the extracellular medium.

DISCUSSION

Secretion by an extrusive process involving fusion of the membranes and channel formation was clearly described for synaptic vesicles by DeRobertis and Bennett in an abstract published in 1954 (6). "Some of the vesicles seem to perforate the presynaptic membrane so that portions of the vesicle lie in the intermembranal space and come into direct contact with the post-synaptic membrane." DeRobertis and Vaz Ferreira (7) found a similar process occurring in the adrenal medulla, and Palade (29) provided ultrastructural evidence for the extrusive secretion of pancreatic zymogen granules. Since 1961, extrusion has been established as a common cellular mechanism for secretion (1, 9, 11–13, 17, 18, 20–22, 27, 31, 34, 37). Several terms, reversed pinocytosis, emiocyto-osis (39), and exocytosis (5), have been suggested for the process; it is the latter that seems to have been sanctioned by widespread use.

The association of mast cell granule dispersal
and histamine release was suggested by Riley (32) and definitively demonstrated by Fawcett (10) who made the important distinction between cytotoxic effects of distilled water and non-lethal effects of the histamine-releasing agent, compound 48/80. Ultrastructural evidence for the maintenance of mast cell integrity during secretion was provided by Thiéry (38) and amply corroborated by subsequent electron microscope (3, 4, 16, 23, 28, 33, 36, 41) and biochemical studies.
FIGURE 10 A possible example of early pore formation at the cell surface. The precise relationship of the membranes at the apparent point of juncture is not clear. \( \times 75,000 \).

(8, 19) of the degranulation process with a range of agents. The possibility has been considered that alterations in granule structure and the expansion of the perigranular space precede the establishment of continuities between the external milieu and the perigranular space (2). The sum of present evidence suggests on the contrary (16, 23, 24, 33) that the alterations depend on the exposure of the granules to extracellular medium which is carried deep into the cell in channels formed by a series of membrane interactions, first between perigranule membranes and the plasma membrane and then between adjacent perigranule membranes. Padawer (28) has raised the possibility that preformed channels, capable of opening and closing, control access of extracellular medium to the granules.

The ultrastructural studies of mast cell secretion to date have not clearly indicated the mechanism of membrane interaction. Röblich et al. (33) have interpreted their electron micrographs as providing evidence for fusion of perigranule and cell membranes with the formation of "a thin, structureless diaphragm bridging the edge of the fused region." They suggest that the formation of the diaphragm is analogous to the formation of the pore diaphragm of fenestrated capillaries as proposed by Wolff and Merker (40). A similar mechanism was previously suggested by Palade and Bruns (30) on the basis of their detailed study of the fusion of pinocytotic vesicles and endothelial cell plasma membrane.

A five-layered membrane with a thickened dense layer where the two unit membranes abut (\( f_{5u} \) of Palade and Bruns) is clearly evident between perigranule membranes during mast cell secretion (Figs. 8 and 9), but I have observed no convincing examples of three-layer membrane fusion (\( f_{3u} \) or of diaphragms lacking unit membrane structure (\( f_{u} \)). Failure to observe images of attenuated intermediates between pentalaminar fusion of two membranes and a pore might be attributable to (a) an exceedingly short lifetime of the intermediate structures, (b) their instability under the conditions of fixation, or (c) their non-existence.1 The rare observation of what appears to be an incipient pore (Fig. 10) offers an alternative to the attenuation model for exocytosis, namely direct pore formation from pentalaminar fused membranes.

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1 Lucy has previously proposed a micellar model for direct pore formation that would not be expected to yield electron microscope images of intermediates beyond the pentalaminar state (25). However, the increasing evidence for the bilayer structure of membranes (14, 35) diminishes the likelihood that Lucy's hypothesis is valid.
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