Surface Morphology of Rat Peritoneal Mast Cells During in vitro Regeneration After Histamine Secretion

Peter Bytzer, Ellen Holm Nielsen, and Jørgen Clausen
Winslow Institute of Human Anatomy, Odense University, Odense, Denmark

Summary. Cell-surface morphology of regenerating mast cells was followed over a period of 48 h after histamine release. Control cells (not stimulated to secrete) were characterized by anastomosing folds of membrane of equal depth and width. During exocytosis these folds disappeared and were replaced by deep cup-shaped flaps of membrane evident in cells incubated for 10 min. During the first hours of regeneration these flaps fused mutually or with the plasma membrane. This activity suggests membrane retrieval, maybe specifically recycling the granule-type patches of membrane. Membrane-fusion activity was observed to some degree also after extended incubation. After 48 h of incubation the regeneration process was still not completed, as indicated by the fact that holes leading to intracellular cavities could still be found.

Key words: Mast cells – Regeneration – Exocytosis – Scanning electron microscopy

In a previous paper we have presented the results of a study on the regeneration process in mast cells after histamine release induced by compound 48/80 as seen with the transmission electron microscope (TEM) (Holm Nielsen et al. 1981). In order to clarify some points related to surface morphology we have undertaken a study of the same regeneration process observed in the scanning electron microscope (SEM).

Previous SEM studies of mast cells have been focused on the cell-surface specializations in relationship to exocytosis (Tizard and Holmes 1974; Kessler and Kuhn 1975; Burwen and Satir 1977) or its prevention by certain anti-allergic drugs (Djaldetti et al. 1979). The present paper is the first report on mast cell recovery after histamine release observed in the SEM.
Materials and Methods

Cell Collection and Incubation

Male Sprague-Dawley rats were killed by bleeding from the carotid arteries under ether anaesthesia and mixed peritoneal cells were obtained by a lavage of the peritoneal cavity with 9 ml of a Krebs-Ringer solution containing 0.05 mg/ml of Heparin (Chakravarty and Echetebu 1978). After 1 min of gentle abdominal massage, the solution was recovered and spun down for 15 min at 300 x g at 37°C. The resulting cell pellet was gently resuspended in 5 ml of Waymouth’s medium and incubated with or without 2 µg/ml compound 48/80 for 2 min in order to induce a secretion. The stimulating effect of compound 48/80 was stopped by diluting the cell suspensions with Waymouth’s medium containing 1 % bovine serum albumin, 100 IU/ml penicillin, 0.1 µg/ml streptomycin, 120 µg/ml anti-PPLO, and 10 mM Hepes to a total of 20–25 ml and a final cell concentration of 30,000–40,000 cells/ml. The samples were aerated with 5 % CO2/95 % atmospheric air and incubated at 37°C in a slowly moving water bath or on a magnetic stirrer. The incubation times were: 10 s, 1 min, 10 min, 2 min, 2 h, 6 h, 12 h, 24 h, and 48 h. For the longest incubation times (48 h) 25 ml of fresh medium was added after 24 h.

Preparation for Scanning Electron Microscopy

The cells were fixed in suspension with an equal volume of 5 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at room temperature for 2 h or over night. They were then spun down, rinsed once in the cacodylate buffer and postfixed in 1 % OsO4 in cacodylate buffer, pH 7.2, for 1 h at room temperature. After rinsing twice, the cells were resuspended in a few drops of buffer and adhered to copperplates coated with Polylsine according to a method described by Mazia et al. (1974). (Poly-L-lysine hydrobromide type 1-B, MW > 70,000, Sigma).

The cells were dehydrated in graded series of acetone or ethanol concentrations, critical point-dried and sputtercoated with Gold/Palladium (20–30 nm). The specimens were studied in a JEOL 100 CX with High-resolution Scanning-attachment at 20 or 40 Kv.

Assessment of Histamine Secretion

The extent of secretion was assessed at the light microscopic level by the intensity of toluidine blue staining of mast cells taken from the samples just after the reaction of compound 48/80 was stopped. In all samples more than 90 % of the mast cells had secreted heavily with a compound exocytosis resulting in small, intracellular, membrane-bound cavities enclosing the non-secreted, faintly stained remnants of the granule matrices. Only a few granules were expelled from the cells. The cell border persisted as a thin cytoplasmic ring.

Results

Incubation for 10 s and 1 min after Stimulation with Compound 48/80 for 2 min

The cells had secreted rather heavily as shown by the many granules still adhering to the surface of the cell (Fig. 1a, b). Extruded granules were very uniform in shape and size, being round and approximately 1 µm in diameter. They were typically slightly porous indicating that they had lost their perigranular membrane during exocytosis (Fig. 3). The cells were ovoid or slightly ellipsoidal with diameters varying from 10 to 20 µm, typically around 14 µm. Between clusters of adherent granules, the surface was dominated by signs of ongoing exocytosis, with flaps of plasma membrane revealing the underlying granules ready for extrusion (Fig. 2).

There was a marked difference between the number of extruded granules from cells incubated for 10 s and from cells incubated for 1 min or longer. On cells incubated for 10 s large surface areas were obviously not yet involved in the exocytosis, their plasma membrane having the appearance of that of control cells,