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Reorganization of the subplasmalemmal cytoskeleton in association with exocytosis in rat mast cells

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Summary. The subplasmalemmal cytoskeleton in mast cells has been studied by scanning electron microscopy of the internal side of the plasma membrane. Rearrangement of the dense subplasmalemmal network of actin filaments took place following cell activation by compound 48/80 and secretion of histamine. The rearrangement was a withdrawal of the subplasmalemmal cytoskeleton from the exocytotic sites and the development of bare, filament-free areas around the sites. In calcium-depleted mast cells we demonstrated a dense network that was difficult to break. Activation of the calcium-depleted cells by compound 48/80 did not induce rearrangement of the network, and in parallel there was no secretion of histamine.

Key words: Mast cell, Cytoskeleton, Exocytosis, SEM, Calcium

Introduction

Secretory cells (Stossel, 1982; Bray et al., 1986) including rat mast cells (Röhlich, 1975; Nielsen and Jahn, 1984; Nielsen and Johansen, 1986) contain a cytoskeleton mainly consisting of actin filaments located close to the plasma membrane. It forms a dense, thick network that may prevent fusion of the granule membrane with the exocytotic site of the plasma membrane (Orci et al., 1977; Hansson et al., 1984; Nielsen and Jahn, 1984; Cheek and Burgoyne, 1986; Nielsen and Johansen, 1986; Aunis et al., 1987). Reorganization or dissembly of the network is likely to be a necessary event in the secretory process as first proposed by Orci et al. (1972), and this seems to be the case in various types of secretory cells (see ref. in Cheek and Burgoyne, 1986). Increased intracellular concentration of calcium ion is essential for breakdown of the rigidity of actin (Bennett, 1984), and elevation of the cytosol calcium concentration may (Sontag et al., 1988), possibly in conjuction with a yet unknown second messenger (Cheek and Burgoyne, 1986; Burgoyne and Cheek, 1987b), trigger the reorganization of the actin network that is necessary for fusion of granule membrane and plasma membrane in exocytosis. Light microscopical investigations and use of immunocytochemistry seem to indicate changes in subplasmalemmal actin in relation to exocytosis in mast cells (Nielsen and Johansen, 1986) and chromaffin cells (Cheek and Burgoyne, 1986). Similarly, in polymorphonuclear leucocytes the filament complex is changed at sites of degranulation, which appear as nude areas on the inner side of the plasma membrane by scanning electron microscopy (Boyles and Bainton, 1981).

The aim of the present investigation was to study the subplasmalemmal cytoskeleton in rat peritoneal mast cells by scanning electron microscopy (SEM) in relation to the secretory process induced by compound 48/80. This was performed by use of isolated cell membranes (Jacobson, 1980). Calcium-depleted mast cells were used to study the calcium dependence of the changes in the subplasmalemmal cytoskeleton.

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Materials and methods

Mixed peritoneal cells (6-14% mast cells) or isolated mast cells (90% mast cells) from male Sprague-Dawley rats, 150-350 g, were obtained by washing of the peritoneal cavity with Krebs-Ringer phosphate buffer, pH 7.2. with 0.05 mg/ml heparine (Johansen and Chakravarty, 1975).

Cells samples were preincubated for 3 h at 37° C in a Krebs-Ringer solution with 12.5 mM Tris buffer, pH 7.2, containing bovine serum albumin, 1mg/ml, 1mM glucose, and 2.5 mM calcium. For the experiments with calcium-depleted mast cells, these were preincubated for 3 h with 5 mM EGTA in a calcium-free medium (White and Pearce, 1983). Then the cells were incubated at 37° C

with compound 48/80, 1-2 μg/ml, in order to activate the exocytosis of histamine. The reaction was stopped after 1 min of incubation by the addition of ice-cold Krebs-Ringer solution.

Cells were attached to Affi-gel 731 beads (Bio-Rad Laboratories) and lysed by vigourously vortexing in a hypoosmolar phosphate buffer (20 mOsm) to reveal the internal side of the fixed part of the cell membrane (Jacobson, 1980). Beads were placed on gelatine-coated strips of alu-foil, fixed for 30 min in 2.5% glutaraldehyde in phosphate buffer, pH 7.2, and post-fixed for 30 min in 1% OsO_4 in water. The specimens were dehydrated in ethanol, critical point dried and coated with 10-12 nm gold/palladium in a cooled sputter coater. From each sample cells were prepared for transmission electron microscopy (TEM). These cells were fixed in the same fixatives for 2 h at 4° C. A few drops of melted agar (37° C) were added to the cell pellet; the agar was chilled on ice and cut into smaller pieces which were dehydrated in ethanol and embedded in Araldite. Thin sections were stained with 1% uranyl acetate and 0.4% lead citrate. TEM and SEM specimens were studied in a Jeol 100 CX electron microscope with scanning attachment. For assesment of secretion, the histamine release was measured by a fluorometric method (Shore et al., 1959). Histamine secretion was calculated as a percentage of the total histamine content of the cells. The spontaneous histamine release after incubation without compound 48/80 has been deducted.

Results

Fig. 1 shows the appearance of the surface of a rat mast cell in SEM. The cell was attached to Affi-gel and was not broken since it had not been through the vortex procedure. The incubation medium contained calcium but not the compound 48/80, and there was no exocytosis of histamine. Incubation with compound 48/80 changed the appearance of the cell surface as demonstrated in SEM in Fig. 2. Histamine secretion induced by compound 48/80 was 64%.

The subplasmalemmal network in the mast cells was shown in SEM of Affi-gel attached cells after lysis of the cells by the vortex procedure performed in hypoosmolar phosphate buffer (Figs. 3, 4). The cells had not been exposed to compound 48/80. The network consisted of tightly packed filaments interconnected at various angles. Through minor openings, 125-225 nm, in the network small patches of the internal side of the plasma membrane could be seen. It is likely that the torn filaments protruding from the network represent filaments which have been connected to the intergranular part of the cytoskeleton (Nielsen and Jahn, 1984).

After incubation of mast cells with compound 48/80 there were exocytotic openings in the plasma membrane. They were rounded in shape and with a size of 300-750 nm (Fig. 5). These openings were surrounded by bare areas revealing the internal side of the plasma membrane. A few filaments were still connected to the plasma membrane in the bare areas. The network outside the exocytotic area was not changed and had a few granules connected to it.

Calcium-depletion seemed to increase the resistance of the mast cells to the vortex procedure. Calcium-depleted mast cells attached to Affi-gel were only partly broken by this procedure, and the cell resistance was not changed by incubation of the cells with compound 48/80 (Fig. 6). Furthermore, after incubation of calcium-depleted cells with compound 48/80, there were few exocytotic openings and the histamine secretion was 5%. As shown in Fig. 7, the subplasmalemmal network in calcium-depleted cells was very dense with small (50-100 nm) openings between the filaments and many granules attached to the filaments. Incubation of the cells with compound 48/80 had no effect on the appearance of the network. TEM of calcium-depleted mast cells after exposure of the cells to compound 48/80 is shown in Fig. 8. This is a normal-looking mast cell, and in parallel with the observation that the secretory response was neligible (5%) only a few granules have been secreted.

Discussion

It has been considered that control of the secretory response may occur through a tow rope mechanism performed by the actin filaments (Bennett, 1984). However, a more likely mechanism seems to be a change; i.e. depolymerization or rearrangement, prior to exocytosis in the subplasmalemmal physical barrier which consists of actin filaments (Allison, 1973; Stossel, 1981; Nielsen and Jahn, 1984; Cheek and Burgoyne, 1986; Nielsen and Johansen, 1986; Burgoyne and Cheek, 1987b; Sontag et al., 1988).

In the present study we have been able to directly examine the morphological changes that follow activation of mast cells with compound 48/80. The experiments

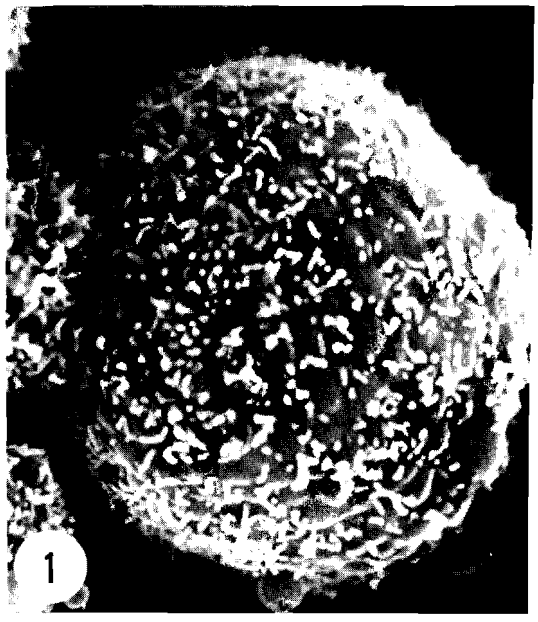

Fig. 1. SEM of non-secreting mast cell. × 5,000

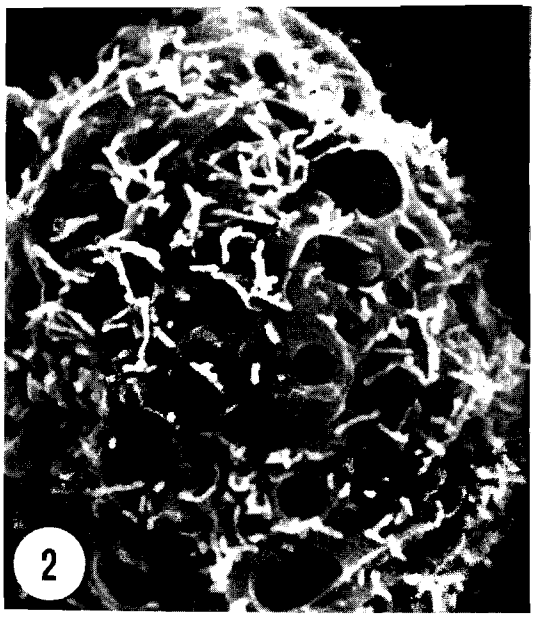

Fig. 2. SEM of secreting mast cell. · 6.000

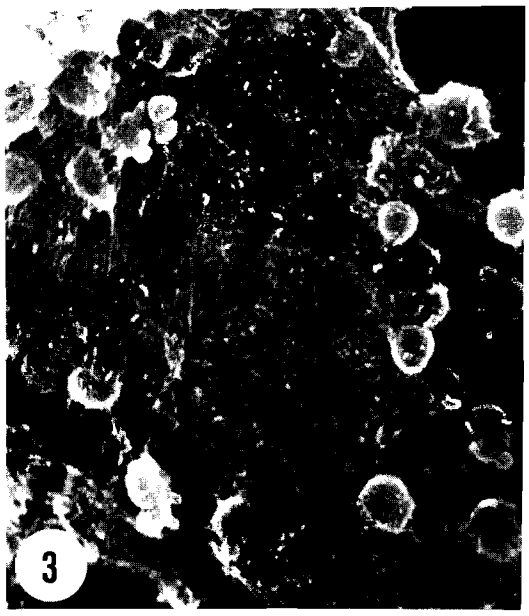

Fig. 3. Attached part of non-secreting mast cell with subplasmalemmal cytoskeleton viewed from inside. × 5,000

Fig. 4. Attached part of non-secreting, vortexed mast cell with subplasmalemmal cytoskeleton viewed form inside. × 15.000

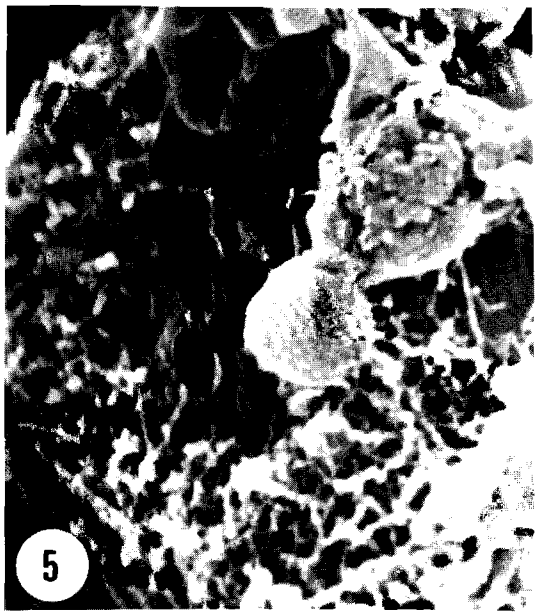

Fig. 5. Attached part of secreting, vortexed mast cell viewed from inside. Exocytotic opening surrounded by filament-free area. × 15,000

were performed with mast cells incubated in presence of calcium as well as with calcium-depleted mast cells. We conclude that a rearrangement of the actin filaments does take place in mast cells following cell activation by compound 48/80. The rearrangement is a withdrawal of the subplasmalemmal cytoskeleton from the exocytotic sites. Around the sites there are bare, filament-free areas of the internal side of the plasma membrane, and this change is likely to be necessary for the fusion between the plasma membrane and the granule membrane. Our findings are consistent with the observations by Boyles and Bainton (1981), who studied endocytosis and exocytosis in polymorphonuclear leukocytes.

The dependence of the rearrangement of the filament network on cytosol calcium (Bennet, 1984; Cheek and Burgoyne, 1986; Burgoyne and Cheek, 1987b) is supported by our observations with calcium-depleted cells. A negligible secretion of histamine in response to compound 48/80 was associated with the finding of a very dense network of filaments that was difficult to break.

While the rearrangement of the network is less likely

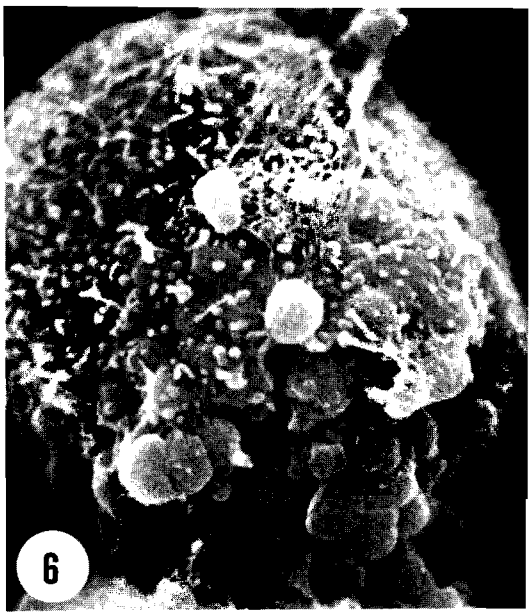

Fig. 6. Vortexed, non-secreting calcium-depleted mast cell. × 5,000

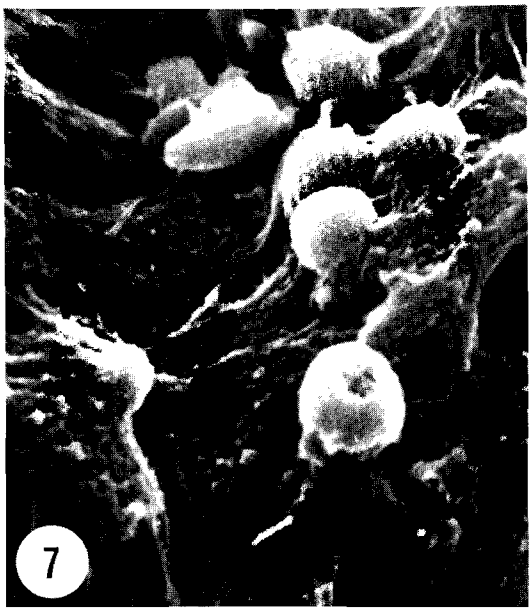

Fig. 7. Attached part of secreting, calcium-depleted mast cell viewed from inside. Subplasmalemmal cytoskeleton is very dense and many granules are still attached to it. × 10,000

to occur through calcium-induced changes in the actin filaments, recent reports suggest that proteins anchoring the cytoskeleton to the membrane may be the target for the secretory signal (Nielsen and Jahn, 1984; Burgoyne and Cheek, 1987a,b; Perrin et al., 1987). This is supported by our observation that the network is nearly completely withdrawn from the exocytotic openings by cell activation.

Since increased cytosol calcium is a likely signal

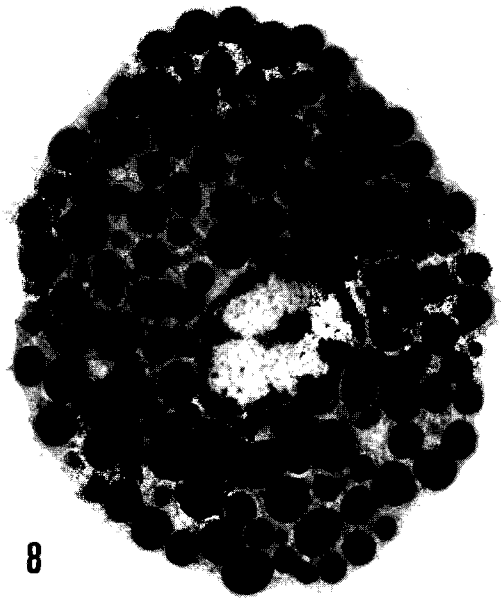

Fig. 8. Section through calcium depleted compound 48/80-incubated mast cell with no secretion. × 3,300. TEM

transducing mechanism, the intracellular calcium-receptor, calmodulin, as well as caldesmon, a granule-binding 70 Kd protein, may be involved in the network rearrangement. Caldesmon binds to calmodulin in a calcium-dependent way, and it has been proposed by Burgoyne et al. (1986) that caldesmon may play a role in regulating the organization of actin filaments of the cell periphery during the secretory process.

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