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Original articles

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MOLECULAR MECHANISM OF HISTAMINE RELEASE: THE ROLE OF INTERMEDIATE FILAMENTS AND MEMBRANE SKELETONS

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It has been recognized that cytoskeletons play some important roles in the histamine release from mast cells. We previously reported the role of microfilaments and microtubules in the histamine release from mast cells, and in the present study, the roles of intermediate filaments and membrane skeletons were investigated. When permeabilized mast cells were stimulated with Ca^{2+}, a translocation of protein kinase C from cytosol to the membrane fraction was observed. This lead to the phosphorylation of vimentin, one of the component proteins of the intermediate filaments. Phosphorylation of vimentin induced disruption of intermediate filaments and resulted in an increase in the mobility of granules. This may be favorable for the initiation of degranulation. In the membrane skeletons of rat mast cells, α- and β-fodrin, ankyrin and actin were found. Changes in the distribution of the fodrin network were elicited by antigen-antibody reaction. It is suggested that membrane skeletons may act as a barrier between the plasma membrane and the granule membrane and that the changes in the distribution of membrane skeletons may facilitate the initiation of the fusion of the plasma membrane and granular membrane.

Key words: *mast cell, histamine release, protein kinase C, intermediate filament, vimentin, membrane skeleton, fodrin, ankyrin, actin.*

INTRODUCTION

It has been shown that cytoskeletons play some important roles in the histamine release from mast cells (1—3). Since cytochalasins, inhibitors of microfilaments, inhibit histamine release from mast cells, it has been supposed that microfilaments participate in the histamine release from mast cells (1, 2). From electron microscopic studies, it was found that various types of

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cytoskeletons exist in rat mast cells set between various organelles including granules and the nucleus as well as between organelles and the plasma membrane (2). As we previously reported, microfilaments may not only push the granules out of the cells but also participate in the cell recovery process after degranulation, retracting the extruded granules inside of the cells (2, 4). On the other hand, since colchicine and vinblastin, inhibitors of microtubules, inhibited histamine release from mast cells, it has been suggested that microtubules are also involved in the histamine release from mast cells (3). Moreover, these inhibitors prevented Ca^{2+} release from the intracellular Ca store of mast cells without affecting the production of inositol 1, 4, 5-trisphosphate (IP3) (3). Actually, electron microscopic observations indicated that microtubules attached themselves to the endoplasmic reticulum, an intracellular Ca store (3, 5). It has also been suggested that microtubules may take part in the histamine release without degranulation (5).

In addition to these two kinds of cytoskeletons, it has been supposed that intermediate filaments and membrane skeletons are important to the cell motility and cell physiology. It has also been suggested that protein kinase C (PKC) may participate in the histamine release from mast cells (6). In the present study, the roles of these two kinds of cytoskeletons in the histamine release from mast cells were investigated.

MATERIALS AND METHODS

Histamine release from permeabilized rat mast cells

Rat peritoneal mast cells harvested from male Wistar rats (300—350 g) were permeabilized with 50 μl of a cytosolic medium (in mM; potassium glutamate 137, $MgSO_4$ 4, adenosine 5′ — triphosphate (ATP) 3, EGTA 5, PIPES 10; pH 6.8) supplemented with 7.5 μg/ml of β-escin at 37°C for 10 min. Subsequently, the medium was replaced by 50 μl of a cytosolic medium containing various concentrations of $CaCl_2$. Thereafter, the cells were incubated for 5 min at 37°C. The reaction was terminated by the addition of 150 μl of an ice-cold Ca^{2+}-free cytosolic medium. The histamine contents released into the supernatant and residual histamine in the cells were determined separately by means of fluorometric assay.

Determination of PKC activity

To determine the total PKC activity of permeabilized cells, 100 μl of a homogenizing buffer (in mM; potassium glutamate 137, 2-mecraptoethanol 10, bovine serum albumin (BSA) 5 mg/ml, phenylmethylsulfonyl fluoride (PMSF) 1, leupeptin 50 μg/ml, EGTA 5,0.1% Triton X-100, PIPES 10; pH 6.8) were added and stirred gently for 30 min on ice. Thereafter, the homogenate was centrifuged at 100,000 × g for 10 min at 4°C. The supernatant was used for the measurement of the total PKC activity.

To determine the PKC activity in the membrane fraction and in the soluble fraction separately, permeabilized cells were incubated in Mg^{2+}- and ATP-free cytosolic medium containing various concentrations of Ca^{2+} at 37°C and the reaction was terminated by placing the plate in an ice bath and by sonicating for 3 sec at 4°C. The sonicated mixture was diluted 2-fold with a medium consisting of (in mM) potassium glutamate 137, 2-mercaptoethanol 20, BSA 10 mg/ml, PMSF 2, leupeptin 100 μg/ml, EGTA 5, PIPES 10 (pH 6.8) and various concentrations of $CaCl_2$ and centrifuged at 100,000 × g for 10 min at 4°C. The supernatant was used as a source of PKC in the soluble fraction, and the pellet was resuspended in 100 μl of homogenizing buffer and placed on ice for 30 min; thereafter, the homogenate was centrifuged at 100,000 × g for 10 min at 4°C. The PKC activity in the supernatant was taken as the PKC activity in the membrane fraction.

The PKC activity was determined by measuring the incorporation of 32P into histone from [γ-^{32P}]-ATP according to the method of Kikkawa et al. (7).

Protein phosphorylation in rat peritoneal mast cells

Purified rat peritoneal mast cells were suspended in phosphate-free Dulbecco's modifield minimum essential medium (107 cells/ml) and incubated with [^{32}P]-orthophosphate (0.5 mCi/ml) for 30 min at 37°C. The cells were washed twice and resuspended in a physiological buffered solution (in mM; NaCl 154, KCl 2.7, $CaCl_2$ 0.9, glucose 5.6, HEPES 5; pH 7.4).

After 5 min of preincubation at 37°C, the cells (106 cells/200 μl) were stimulated with 50 μl of compound 48/80. The reaction was terminated by adding 50 μl of 3 × concentrated SDS-PAGE sample buffer (9% SDS, 150 mM Tris-HCl, 3% 2-mercaptoethanol, 15% glycerol; pH 6.8). The cellular proteins were further denaturated in boiling water for 5 min. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a slab gel with a 4—20% gradient of polyacrylamide according to the method of Laemmli (8). After electrophoresis, autoradiography of the gel was carried out using Hyperfilm (Amersham) for 3 days at −80°C. The autoradiograms were scanned using a densitometer (Bio-Rad) to quantify the relative radioactivity.

Immunoprecipitation of phosphorylated proteins

After rat mast cells were stimulated with compound 48/80, the reaction was terminated by the addition of radioimmunoprecipitation assay (RIPA) buffer (in mM; NaCl 300, NaF 100, sodium pyrophosphate 20, sodium orthovanadate 5, EDTA 5, PMSF 2, Tris-H_3PO_4 100, pH 7.5, 2% Triton X-100, 0.2% SDS, 0.2% sodium deoxycholate). After vortex mixing, the mixture was centrifuged at 100,000 × g for 5 min at 4°C. The supernatant was incubated with 1/10th volume of antibody solution (either anti-vimentin, anti-desmin, anti-tubulin antibody or normal mouse serum) for 1 hr at 4°C, and then 20 μl of a protein A-cellulofine bead suspension were added. The mixture was shaken continuously for 1 hr at 4°C and sedimented at 100,000 × g for 5 min at 4°C. The pellet was washed three times with RIPA buffer and 50 μl of SDS-PAGE sample buffer were added. SDS-PAGE analysis and autoradiography was carried out as described above.

Immunofluorescent microscopy of vimentin

Rat mast cells placed on a cover glass were stimulated with 0.5 μl/ml of compound 48/80 for various periods of time and the cells were fixed with 3.7% formaldehyde in PBS containing 1 mM $MgCl_2$ for 30 min, and then treated with 0.1% Triton X-100 for permeabilization. After the blocking of non-specific binding sites, the fixed cells were incubated with anti-vimentin mouse IgG (1:10) for 3 hr at room temperature. Thereafter, FITC-conjugated anti-mouse IgG (1:1000) was applied to the cells for 1 hr. The fluorescence intensity of the stained cells was measured by means of a photon counter connected to a fluorescence microscope.

Observation of the subplasmalemmal region of mast cells by means of scanning electron microscope

Rat peritoneal mast cells were incubated on a collagen-coated cover glass at 37°C for 30 min, and the cells were fixed with 0.5% glutaraldehyde and 3% formaldehyde. Thereafter, the cells were permeabilized with 0.1% Triton X-100 at 37°C for 5 min. After the blocking of non-specific binding sites, the cells were incubated with anti-α-fodrin mouse IgG, and subsequently incubated with anti-mouse IgG conjugated with colloidal gold (15 nm). The cells were post-fixed with 2.5% OSO4, dehydrated with graded ethanol series and critical-point dried. Dry-cleaving of the specimen was carried out according to the method of Mesland et al. (9). The specimens were coated with carbon and examined with a scanning electron microscope (Hitachi S-800) connected to an X-ray microanalyzer (Kevex).

Western blotting analysis of membrane skeletal proteins

Rat mast cells were disrupted by a sonication for 5 sec at 4°C in HEPES (5 mM)-buffered sucrose solution. After centrifugation at 3,000 × g for 20 min to sediment debris and nondisrupted cells, the supernatant was centrifuged at 100,000 × g for 1 hr. The pellet (membrane fraction) was suspended in SDS-PAGE sample buffer and SDS-PAGE analysis was carried out. Thereafter, the separated proteins in the gel were transferred to the nitrocellulose membrane using a dry-blotting apparatus. The nitrocellulose membrane was incubated in an antibody solution (either anti-α-fodrin, anti-β-fodrin or anti-ankyrin) and detection of the protein band was carried out using ECL-Western system (Amersham).

Immunofluorescence microscopy of membrane skeletons

Rat mast cells incubated on a collagen-coated cover glass were fixed with 0.5% glutaraldehyde and 3% formaldehyde and permeabilized with 0.1% Triton X-100. After the blocking of non-specific binding sites, the cells were incubated with anti-α-fodrin mouse IgG, and subsequently, incubated with anti-mouse IgG conjugated with FITC simultaneously with rhodamine-phalloidin. After that, the cells were examined with a fluorescence microscope. In some cases, sensitized mast cells were stimulated with anti-IgE conjugated with colloidal gold (40 nm) and polarization microscopy was also carried out.

RESULTS

ROLE OF INTERMEDIATE FILAMENTS IN THE HISTAMINE RELEASE FROM MAST CELLS

Translocation of PKC from cytosol to the membrane fraction

When permeabilized mast cells were stimulated with various concentrations of Ca^{2+}, histamine release took place in a concentration-dependent manner (*Fig. 1*). However, the PKC activity in the cells determined simultaneously with histamine release was in no way affected by Ca^{2+}. This may suggest that Ca^{2+} does not affect the total PKC activity in the cells.

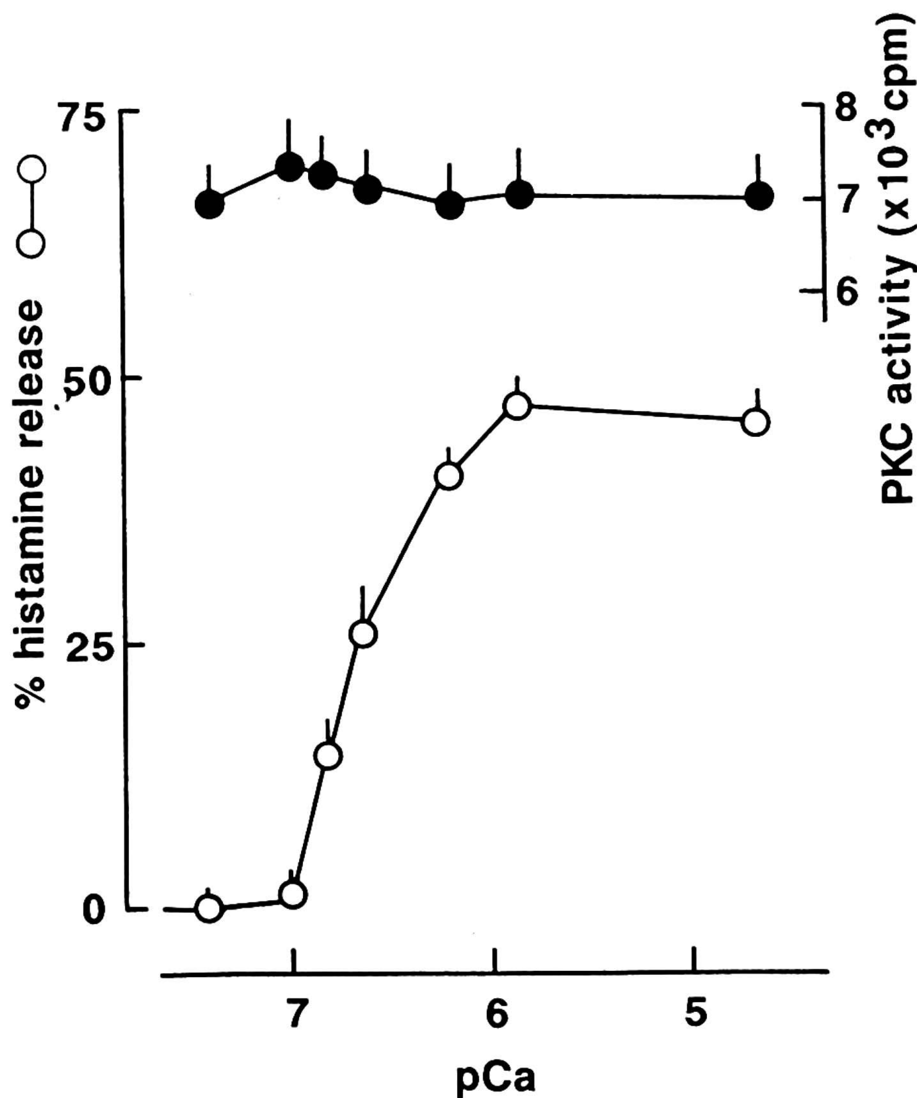

Fig. 1. Histamine release (O) and the total PKC activity (●) in β-escin-permeabilized mast cells induced by Ca^{2+}. Permeabilized mast cells were incubated in an intracellular medium containing various concentrations of Ca^{2+} for 5 min at 37°C. Each point represents the mean + S.E.M. ($n = 4$). * and ** represent the statistical significance in $p < 0.05$ and $p < 0.01$, respectively.

On the other hand, after stimulation of permeabilized mast cells with various concentrations of Ca^{2+}, the PKC activities in both soluble and insoluble fractions were determined separately. The activity of PKC in the Triton-soluble fraction decreased and, in relation to this, the PKC activity in the Triton-insoluble fraction increased in a concentration-dependent manner with Ca^{2+} (*Fig. 2*). From these results, it was supposed that an increase in the Ca^{2+} concentration after the application of histamine-releasing stimuli induced a translocation of PKC from cytosol to the membrane fraction. Since cytoskeletons are known to be insoluble in Triton X-100, it was supposed that the site of action of PKC may be the cytoskeletons. The following studies were carried out in order to identify the substrate of PKC in the cytoskeletons of mast cells.

Phosphorylation of vimentin due to PKC

When rat mast cells were stimulated with compound 48/80, a marked phosphorylation was elicited at 59 and 45 kDa proteins (*Fig. 3*). Although 45 kDa protein was found only in the Triton-soluble fraction, 59 kDa protein was

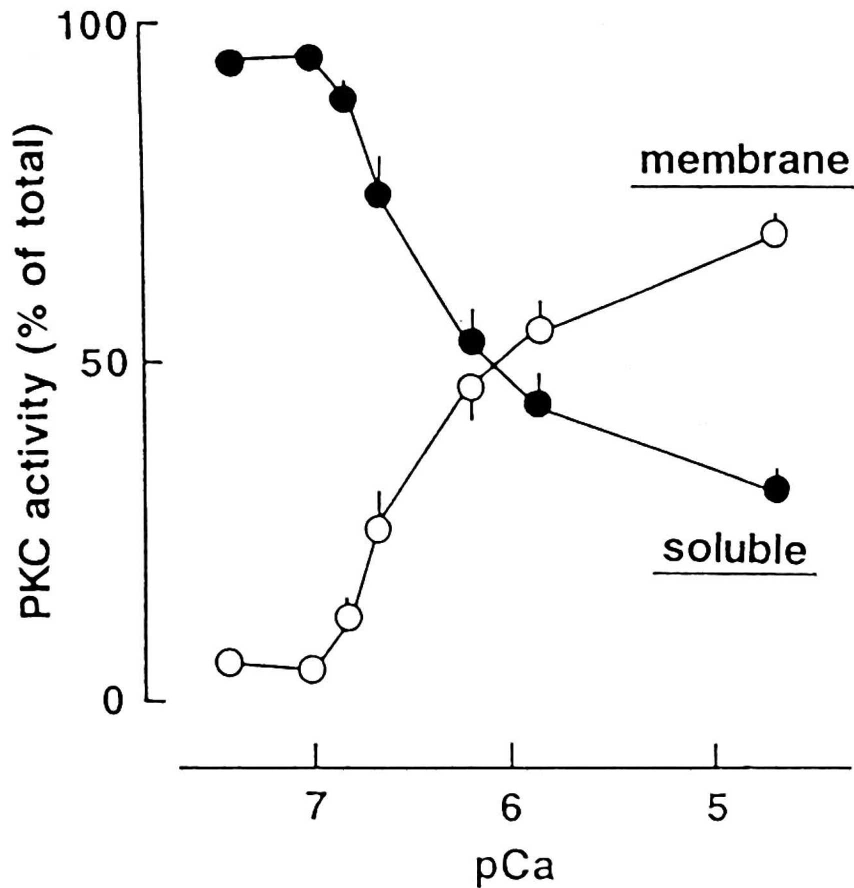

Fig. 2. Translocation of PKC activity from soluble fraction to membrane fraction induced by Ca^{2+}. O: PKC activity in the membrane fraction; ●: PKC activity in the soluble fraction. Permeabilized mast cells were incubated for 5 min in a Mg^{2+}/ATP-free intracellular medium containing various concentrations of Ca^{2+}. The PKC activity was expressed as percentages of the total PKC activity. Each point represents the mean+S.E.M. ($n = 4$). * and ** represent the statistical significance in $p < 0.05$ and $p < 0.01$, respectively.

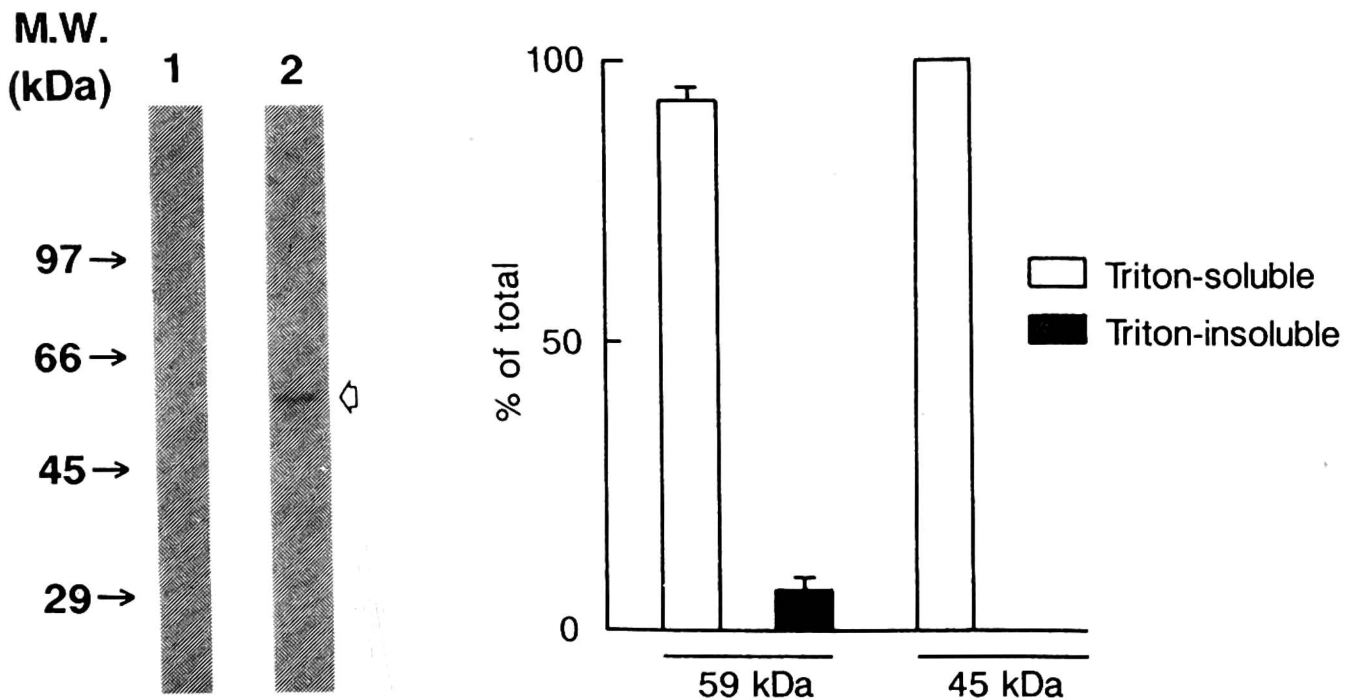

Fig. 3. Phosphorylated proteins of rat mast cells in Triton-soluble and insoluble fractions. The left panel indicates an autoradiograrn of the phosphorylated protein in Triton-insoluble fraction in rat mast cells stimulated by compound 48/80 (0.5 μg/ml). Lane 1: control. Lane 2: compound 48/80. The right panel indicates a distribution of 59 and 45 kDa phosphorylated proteins in rat mast cells stimulated by compound 48/80 (0.5 μg/ml). Each column represents the mean±S.E.M. ($n = 3$).

found in both the Triton-soluble and insoluble franctions. Since phosphorylation of 59 kDa protein was found in the Triton-insoluble fraction, this protein appeared to be a cytoskeletal protein.

Immunoprecipitation analysis of mast cell proteins after stimulation with compound 48/80, has revealed that the phosphorylated 59 kDa protein was vimentin, which is one of the components of intermediate filaments (*Fig. 4*). Neither anti-tubulin antibody nor anti-desmin antibody reacted with this protein. From these results, it was inferred that phosphorylation of vimentin in mast cells takes place after stimulation with compound 48/80.

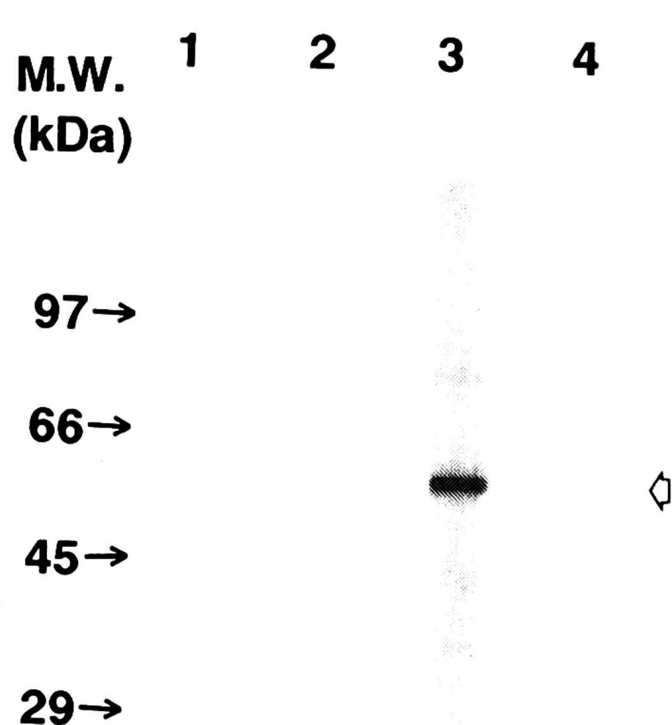

Fig. 4. Immunoprecipitation analysis of 59 kDa phosphorylated protein in rat peritoneal mast cells induced by compound 48/80 (0.5 μg/ml). Lane 1: normal mouse serum. Lane 2: anti-desmin antibody. Lane 3: anti-vimentin antibody. Lane 4: anti-tubulin antibody.

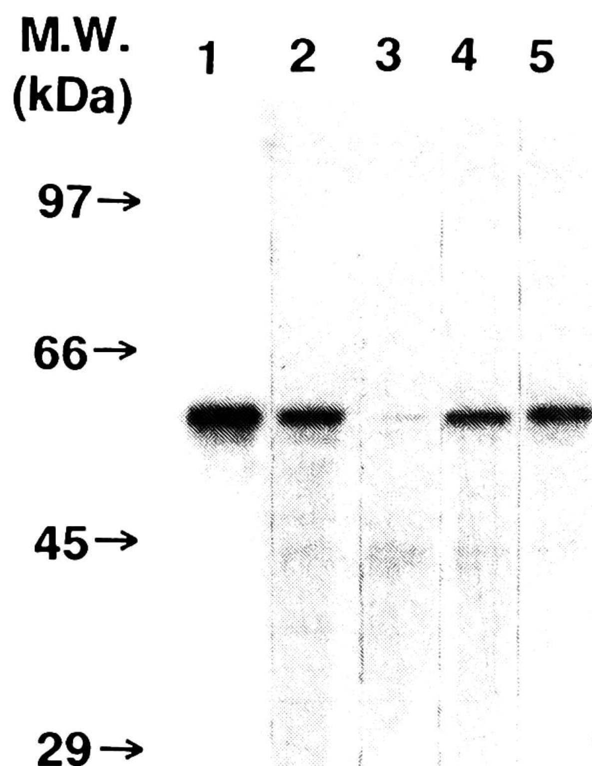

Fig. 5. Effects of calphostin C and calmodulin inhibitors on vimentin phosphorylation in permeabilized rat mast cells. Lane 1: control ($+Ca^{2+}$). Lane 2: calphostin C (0.5 μM). Lane 3: calphostin C (1 μM). Lane 4: trifluoperazine (10 μM). Lane S: W-7 (25 μM).

Since it was supposed that vimentin phosphorylation took place under the condition which induces an activation of PKC, the effect of protein kinase inhibitors on the compound 48/80-induced vimentin phosphorylation was studied. Although calmodulin inhibitors, such as W-7 and trifluoperazine, did not affect the phosphorylation of vimentin, the PKC inhibitor, calphostin C, potently inhibited the phosphorylation of vimentin (*Fig. 5*). This result clearly demonstrates that an activation of PKC after compound 48/80-stimulation induces the phosphorylation of vimentin.

Participation of vimentin phosphorylation in the histamine release from mast cells

The time courses of histamine release and vimentin-phosphorylation in rat peritoneal mast cells elicited by compound 48/80 were determined simultaneously. After compound 48/80 stimulation, the phosphorylation of vimentin took place parallel to histamine release from mast cells, indicating that vimentin phosphorylation concurrently takes place with histamine release from mast cells (*Fig. 6*).

When normal rat mast cells stained with anti-vimentin antibody conjugated with FITC were observed by means of a fluorescence microscope, the

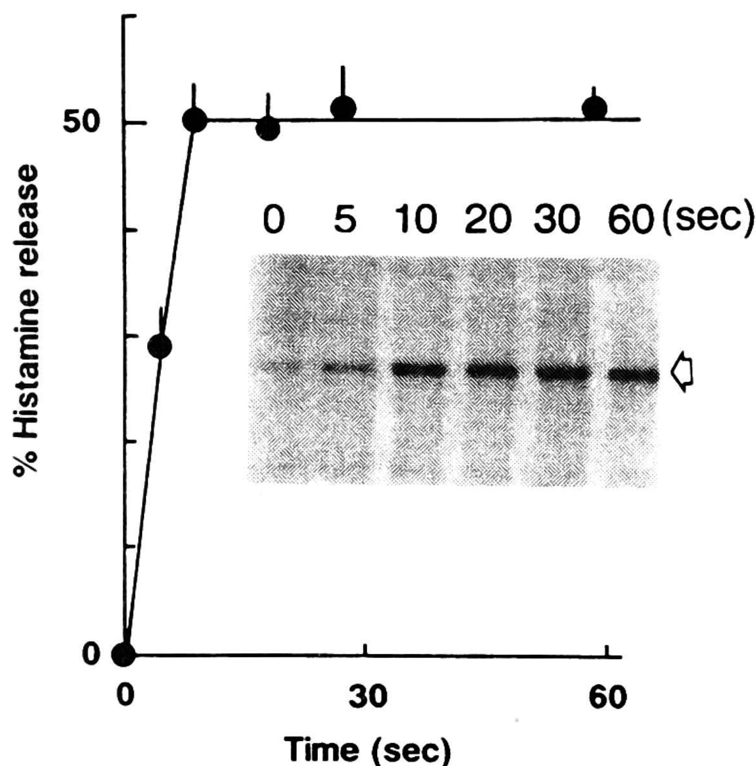

Fig. 6. Time courses of the histamine release and vimentin phosphorylation in rat mast cells induced by compound 48/80 (0.5 μg/ml).

network structure of intermediate filaments was observed inside of the cells. On the other hand, when mast cells were stimulated by compound 48/80, the vimentin filaments were disrupted. The fluorescence intensity of the stimulated cells was weaker than that of the control cells. When the changes in the fluorescence intensity of mast cells stained with anti-vimentin antibody conjugated with FITC were measured after stimulation with compound 48/80, it was found that the fluorescence intensity rapidly decreased after an addition of compound 48/80. This result indicates that depolymerization of vimentin took place after stimulation of mast cells with compound 48/80. When the mast cells were stained with anti-vimentin antibody conjugated with colloidal gold and observed by means of immunoelectron microscopy, it was found that the filamentous structure of the intermediate filaments connecting the granules and other organelles was densely surrounded with immunogold, indicating that these filaments are actually vimentin.

ROLE OF MEMBRANE SKELETONS IN THE HISTAMINE RELEASE FROM MAST CELLS

Scanning electron microscopic observation of the subplasmalemmal region of mast cells

When the subplasmalemmal region of rat peritoneal mast cells was observed by means of SEM, complicated network structures of cytoskeletons with various thicknesses were observed. Such filamentous structures are known as membrane skeletons. Although it has been recognized that membrane skeletons play some important roles in the cell motility as well as the movement of membrane proteins in the cell membrane (10), little is known about the membrane skeleton of mast cells, morphologically as well as biochemically.

Western blotting analysis of membrane skeletal proteins

In order to study the components of membrane skeletons, western blotting analysis of mast cell membrane fractions was carried out. α-Fodrin was detected as 240 kDa protein and β-fodrin was observed as 235 kDa protein in mast cell membrane fraction (*Fig. 7*). This results was in good agreement with the fact

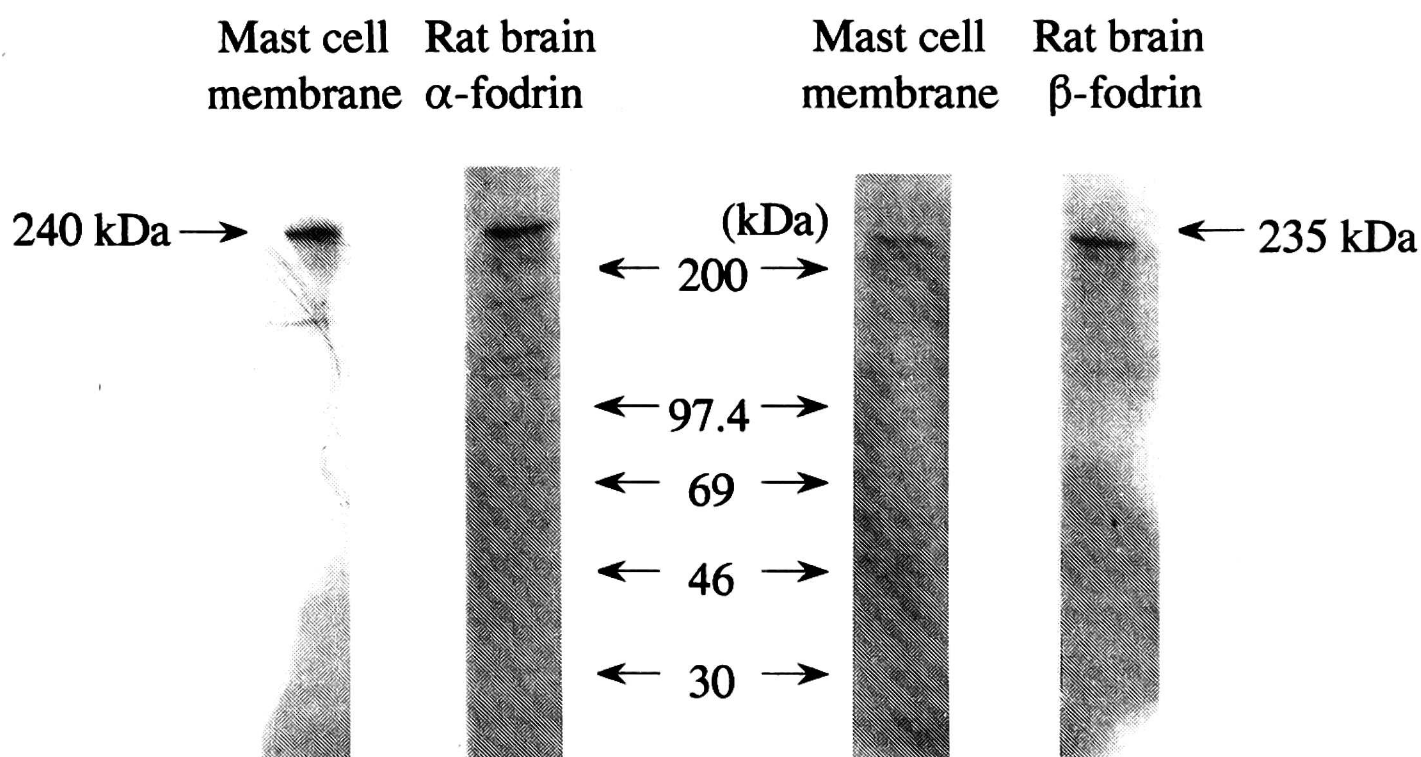

Fig. 7. Western blotting analysis of α- and β-fodrin in rat peritoneal mast cells.

α- and β-fodrin form a heterodimer which makes up the membrane skeletons as reported in other kinds of cells (10). In addition to this, ankyrin was also detected as 220 kDa protein in the mast cell membrane by means of western

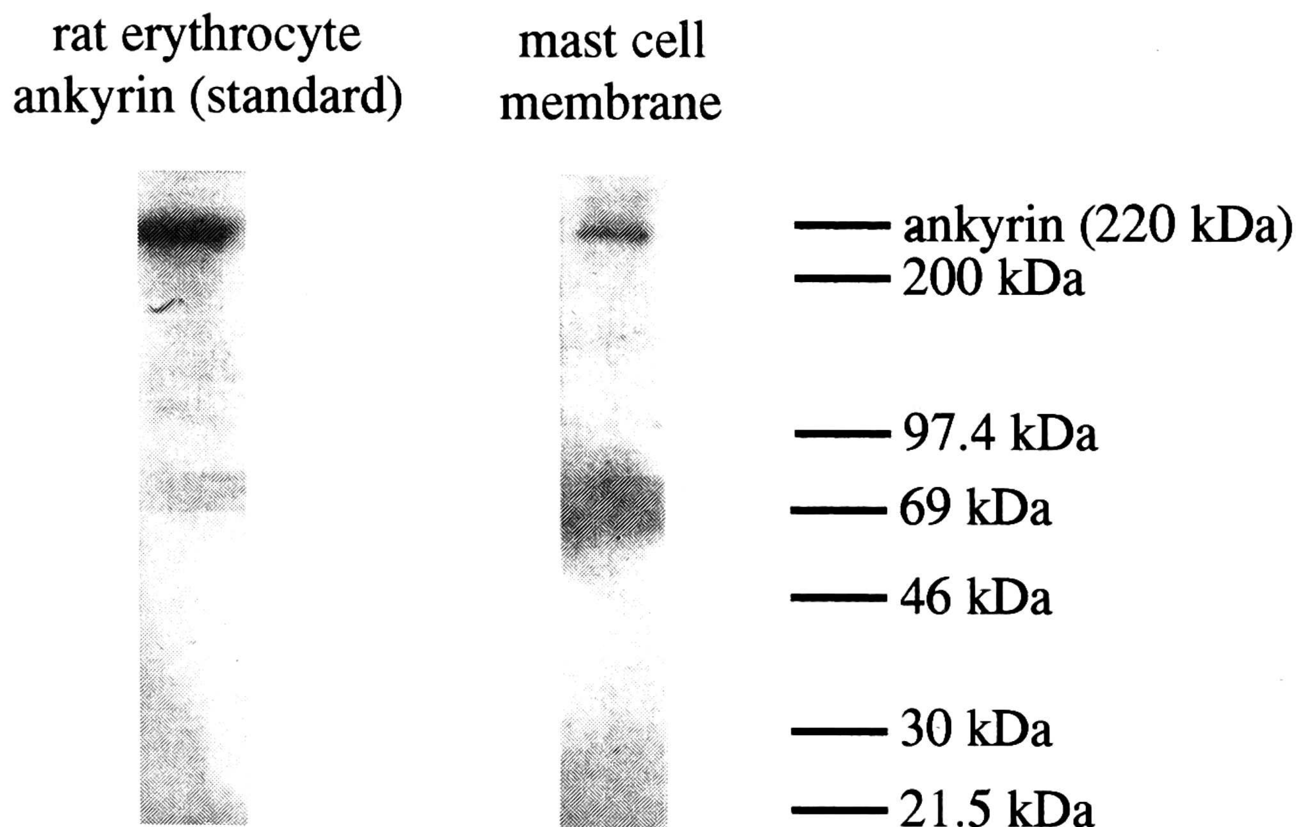

Fig. 8. Western blotting analysis of ankyrin in rat peritoneal mast cells.

blotting analysis, indicating that the membrane skeleton of mast cells also contains ankyrin as in the case of other kinds of cells (*Fig. 8*).

Immunoelectron microscopic observation using X-ray microanalysis

In order to study the distribution of fodrin in the membrane skeleton of mast cells, the subplasmalemmal region of mast cells was labeled with anti-α-fodrin antibody conjugated with colloidal gold (15 nm). Thereafter, the specimen was observed with SEM connected to an Xray microanalyzer, and the characteristic X-ray irradiated from the L-shell of gold atoms was detected. The distribution of irradiated X-ray from gold atom was superimposed on the SEM image of subplasmalemmal network using an image processor. Gold particles conjugated with anti-α-fodrin antibody distributed along with the filamentous structures of the membrane skeleton, indicating that the fodrin network is one of the major components of the membrane skeleton of mast cells (*Fig. 9*).

Immunofluorescence microscopic observations of membrane skeletons

Since it has been suggested that, in several kinds of cells, the fodrin network is intimately connected to actin filaments (10), changes in the distribution of the fodrin network and actin filaments in mast cells were simultaneously examined by means of immunofluorescence microscopy. In this case, both rat peritoneal

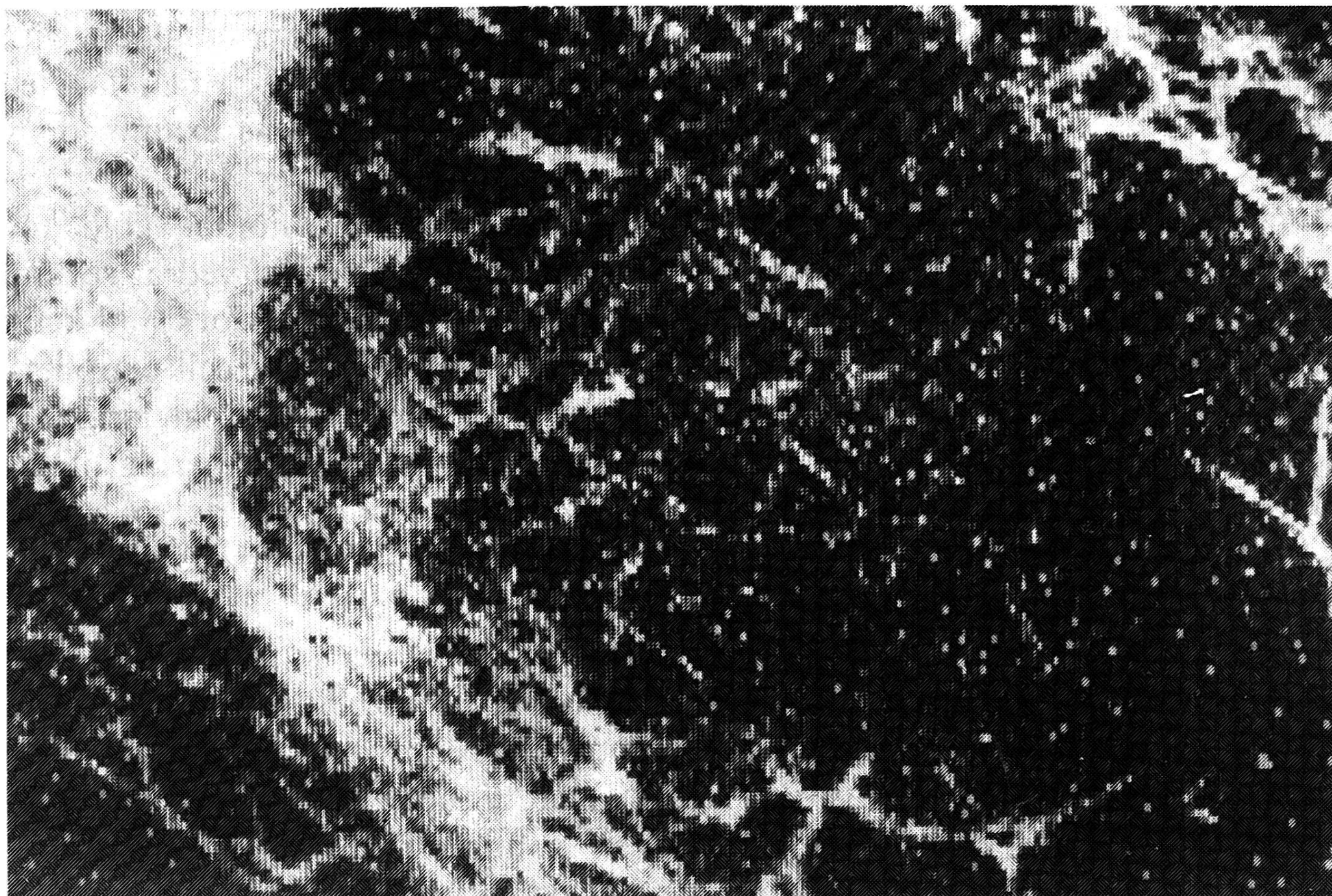

Fig. 9. Immunoelectron microscopic observation using X-ray microanalysis The subplasmalemmal region of mast cells was labeled with anti-α-fodrin antibody conjugated with colloidal gold (15 nm). Thereafter, the specimen was observed with SEM connected to an X-ray microanalyzer, and the characteristic X-ray irradiated from the L-shell of gold atoms was detected. The distribution of irradiated X-ray from gold atom (yellow dots) was superimposed on the SEM image of subplasmalemmal network using an image processor.

mast cells and macrophages were stained with both FITC-conjugated anti-α-fodrin antibody and with rhodamine-phalloidin, which is capable of binding specifically to actin filaments.

In the case of mast cells, a filamentous network of fodrin was observed on the cell surface. On the other hand, the distribution of actin filaments on the cell surface was similar to those of microfolds seen on the surface of mast cell membranes (2). Although the distribution of fodrin was similar to that of actin tilaments on the whole, slight differences were noticed, especially in the microfolds.

In contrast to this, when rat peritoneal macrophage was stained similarly, the distribution of fodrin and actin was quite different from those seen in mast cells. Fluorescence derived from fodrin was densely gathered in the center of the cell, while actin filaments were distributed throughout the whole cell, even in the cell periphery. Stress fiber, consisting of actin filaments, was clearly observed in pseudopodium.

In order to study the changes in the distribution of membrane skeletons in association with histamine release, isolated rat peritoneal mast cells were

sensitized with IgE and the cells were exposed to anti-IgE conjugated with colloidal gold. Thereafter, the cells were stained with FITC-labeled anti-α-fodrin and rhodamine-phalloidin, and the specimen was examined by means of a fluorescence-polarization microscope.

When sensitized rat mast cells were exposed to colloidal gold (40 nm)-conjugated anti-IgE, a patching of gold particles was observed at several places on the mast cell surface. In this case, both the fodrin network and actin filaments were disrupted. However, fluorescence intensity at the cell periphery of FITC-stained cells increased, especially at the site close to the patching and degranulation. This may suggest that the redistribution and gathering of the fodrin network take place after antigen-antibody reaction so as to ensure a favorable consition for exocytosis. The patching of Fcε receptors may also participate in the redistribution of the fodrin network.

In the case of the spectrin of erythrocytes, it has been pointed out that membrane-bound Ca may be useful in stabilizing the structure of the spectrin network. Since fodrin is an analog of spectrin and known as non-erythrocyte spectrin, it is useful to clarify the role of membranebound Ca in the fodrin network. This was examined by means of immunofluorescent microscopy.

Rat peritoneal mast cells were permeabilized with β-escin treatment and the cells were exposed to a Ca-free medium containing 0.1 mM EGTA. Thereafter, the cells were stained as described previously. When permeabilized mast cells were treated with the Ca-free medium containing EGTA, the fluorescence intensity of fodrin, derived from FITC-conjugated antifodrin antibody, dramatically decreased, suggesting that Ca-depletion induced disruption of the fodrin network. On the other hand, the distribution of actin filaments was not significantly affected by this treatment.

DISCUSSION

Microfilaments consist of mainly actin and form the connections between organelles including granules and nuclei. The filaments may be useful in pushing or squeezing granules out of the cells (2, 4). Microtubules consist of tubulin and MAPs. Microtubules located near the endoplasmic reticulum seem to play an important role in the regulation of Ca^{2+} release from the endoplasmic reticulum (3, 5). Furthermore, it was supposed that microtubules may play some roles in the process of histamine release without the accompanying degranulation (5).

As indicated in the present study, when the intracellular Ca^{2+} concentration of mast cells was increased, translocation of PKC from cytosol to membrane fractions occurred. Since the membrane fraction employed in the present study contains cytoskeletal proteins, it was supposed that cytoskeletal

protein would be a possible candidate for the target of PKC. Actually, phosphorylation of 59 kDa protein was observed in the Triton-insoluble fraction, which exclusively contains the cytoskeletons (*Fig. 3*). Immunoprecipitation analysis revealed that the phosphorylated 59 kDa protein is vimentin, one of the components of intermediate filaments, as shown in *Fig. 4*. Since the phosphorylation of vimentin was inhibited by the presence of calphostin C, a specific PKC inhibitor, it became clear that one of the sites of action of PKC in mast cells is vimentin. From immunocytochemical analysis, it was indicated that intermediate filaments, containing vimentin, exist in the perigranular spaces connecting granules. Since the fluorescence intensity of mast cells stained with anti-vimentin antibody conjugated with FITC decreased after compound 48/80-stimulation, it was assumed that the disruption of intermediate filaments took place after compound 48/80-stimulation as a consequence of vimentin phosphorylation. Since vimentin filaments exist in perigranular spaces and surround the granules, the disruption of vimentin filaments induced by phosphorylation may be a good reason for the granule movement toward the cell membrane, and this can be a proper trigger for the degranulation.

It has been shown that membrane skeletons play a variety of important roles in the cell motility as well as in the movement of membrane proteins in the cell membrane (10). In addition, it has been reported that band 3 protein, which is a Con A-binding protein in erythrocytes, attaches to the spectrin network and that lateral movement of band 3 protein may be regulated by spectrin networks (10). The importance of fodrin has been emphasized in the exocytosis of chromaffin cells and platelets (11, 12). However, the actual evidence that the movement of membrane proteins is intimately related with the fodrin network had not been obtained. Furthermore, and most critically, it is unknown whether or not fodrin exists in mast cells.

From the western blotting analysis, it was revealed that the membrane skeleton of rat peritoneal mast cells contains α-fodrin, β-fodrin and ankyrin (*Fig. 7—9*). Furthermore, since rhodaminephalloidin also bound at the subplasmalemmal region of mast cells, it was strongly assumed that actin filaments exist in the membrane skeleton of mast cells, as in the cases of several other types of cells (10). The changes in the distribution of membrane skeletons elicited by antigenantibody reaction were remarkable. From the observations obtained by means of immunoelectron and immunofluorescence microscopies, it became clear that fodrin is located in the membrane skeleton in a complicated network connected to actin filaments. It is also suggested that the lateral movement of the Fcε receptors on the mast cell membrane may be intimately related to the changes in the shape of the fodrin network which exists under the cell membrane and that when the cells are stimulated, either with compound 48/80 or with antigen, rearrangement of the fodrin network

may cause the lateral movement of membrane proteins, which would increase the chance of the granules making contact with the cell membrane which in turn would finally result in degranulation. In fact, it was supposed that membrane skeletons may act as a barrier separating the plasma membrane and granule membrane at the resting stage, and that the changes in the distribution of membrane skeletons may be crucial in inducing the initiation of the fusion of plasma membrane and granular membrane which is the initial stage of degranulation.

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