Regulatory Interaction of N-Formyl Peptide Chemoattractant Receptors with the Membrane Skeleton in Human Neutrophils

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The cytoskeleton and/or membrane skeleton has been implicated in the regulation of N-formyl peptide receptors. The coupling of these chemotactic receptors to the membrane skeleton was investigated in plasma membranes from unstimulated and desensitized human neutrophils using the photoreactive agonist N-formyl-met-leu-phe-N-\(^2\)p-azidosalicylamidoethyl-1,3'-dithiopropionate (fMLFK-\(125\)I)ASD. When membranes of unstimulated cells were solubilized in Triton-X 100, a detergent that does not disrupt actin filaments, only 50% of the photoaffinity-labeled receptors were solubilized sedimenting in sucrose density gradients at a rate consistent with previous reports. The remainder were found in the pellet fraction along with the membrane skeletal actin. Solubilization of the membranes in the presence of p-chloromercuriphenylsulfonic acid, elevated concentrations of KCl, or deoxyribonuclease I released receptors in parallel with actin. When membranes from neutrophils, desensitized by incubation with fMLFK-\(125\)IASD at 15°C, were solubilized, nearly all receptors were recovered in the pellet fraction. Incubation of cells with the ligand at 4°C inhibited desensitization partially and prevented the conversion of a significant fraction of receptors to the form associated with the membrane skeletal pellet. In these separations the photoaffinity-labeled receptors not sedimenting to the pellet cosedimented with actin. Approximately 25% of these receptors could be immunosolated with antitoxin antibodies suggesting that N-formyl peptide receptors may interact directly with actin. These results are consistent with a regulatory role for the interaction of chemotactic N-formyl peptide receptors with actin of the membrane skeleton. *Journal of Immunology*, 1994, 152: 801.

Stimulation of FPR\(^1\) on human neutrophils induces a variety of host defensive functions including superoxide production, adhesion, chemotaxis, and secretion (1, 2). These receptors transduce signals via pertussis toxin-sensitive guanyl nucleotide-binding proteins (G proteins) that activate phospholipase C. Two major pertussis toxin substrates have been identified in human neutrophils and HL60 cells, i.e., G\(_2\) and G\(_3\) (3–5). Functional interaction with FPR has been demonstrated for both G\(_2\) subtypes (6). In addition, physical interaction of FPR with G\(_\alpha\), a G proteins isolated from human neutrophils (7) that is probably identical to G\(_{12}\), or G protein from bovine brain (G\(_{12}\) and G\(_{13}\)) has also been shown in a recently developed reconstitution assay that includes velocity separation of receptors and receptor-G protein complexes on detergent-containing sucrose density gradients (8).

For several G protein-coupled receptors, e.g., \(\beta\)-adrenergic and muscarinic receptors, the phenomenon of desensitization in which the cells become insensitive to agonists has been observed (9, 10). Several mechanisms of desensitization have been proposed. These include 1) receptor phosphorylation, which increases the affinity of a receptor for arrestinlike proteins (11) and ultimately leads to uncoupling of receptors from their specific G protein; 2) receptor sequestration in which the access of ligand to the receptors is restricted; and 3) receptor down-regulation in which the receptor number of the cell is diminished. Desensitization with respect to FPR-mediated superoxide production is observed in neutrophils after prolonged exposure of the cells to an agonist (12). Although phosphorylation of FPR has not been detected (13), it cannot be

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\(^{1}\) Abbreviations used in this paper: FPR, N-formyl peptide receptor(s); dBCB, dihydrocyclobasealin B; DNase I, deoxyribonuclease I; G protein, guanyl nucleotide-binding protein; OG, in octyl-\(\beta\)-d-glucopyranoside; pCMPS, p-chloromercuriphenylsulfonic acid; TX-100, Triton X-100; WGA, wheat germ agglutinin.
ruled out as a possible regulatory mechanism in this process. We have hypothesized that receptor segregation from G protein in the plasma membrane may be a critical event in the desensitization process of FPR. It may also be a unique mechanism for this receptor or for chemotactic receptors in general (14).

Several lines of evidence point to an important role of the cytoskeleton and/or the submembranous membrane skeleton in the regulation of FPR sensitivity. First, the transient chemotactic peptide-induced superoxide production can be prolonged in the presence of dhCB, an alkaloid that disrupts microfilaments (15). Second, FPR in desensitized cells accumulate in the TX-100-insoluble fraction (16, 17). Finally, FPR in desensitized cells also accumulate in an actin- and fodrin-rich domain of the plasma membrane that is depleted of G proteins (18). Taken together, these data suggest that coupling of receptors to the cytoskeleton and/or membrane skeleton might possibly serve as a mechanism to segregate receptors and G proteins into different domains of the plasma membrane and thus physically interrupt the signaling cascade. A mechanism like this would require a physical interaction of FPR and the cytoskeleton and/or membrane skeleton. However, the molecular basis for such coupling has not been described.

The association of receptors with cytoskeletal structures has been suggested in various systems including β-adrenergic receptors (19), glycoprotein IIa/III (20), hyaluronate receptors (21), IFN-α receptors (22), and IgE receptors (23), to name a few. Several of these reports point to a possible regulatory role for receptor-cytoskeleton and/or membrane-skeleton interactions.

Erythrocytes contain a two-dimensional membrane-associated actin network rather than a three-dimensional structure throughout the cytoplasm (24). Such a membrane skeleton (25, 26) has meanwhile been identified in many other cells like platelets (27), liver cells (28), and leukocytes (29). We attempted, therefore, to characterize the interaction of FPR with the membrane skeleton in isolated plasma membranes from human neutrophils. We found that a certain fraction of these receptors in unstimulated cells sedimented as TX-100-insoluble complexes in detergent-containing sucrose density gradients, whereas virtually all receptors are complexed in desensitized cells. The receptor linkage to the membrane skeleton paralleled the supramolecular state of actin suggesting a direct or indirect interaction of FPR with actin filaments.

Materials and Methods

Monoclonal antiactin antibodies (lGM) for Western blots were purchased from Amersham, Arlington Heights, IL. For immunodensitometry studies rabbit antiantiin serum from ICN Biomedicals, Inc., Costa Mesa, CA, was used. Goat anti-mouse IgM (alkaline phosphatase conjugate) was from Bio-Rad, Richmond, CA. TX-100 and guanly nucleotides were from Boehringer Mannheim, Indianapolis, IN, and octylglucoside was from Calbiochem, La Jolla, CA. PCMPS was obtained from Sigma, St. Louis, MO. All other materials were from sources previously described (18, 30). Procedures for SDS-PAGE and Western blot analysis have been conducted as described (18).

Cells and membranes

Human neutrophils have been prepared by a gelatin sedimentation procedure as described recently (31). Desensitized neutrophils were prepared by photoaffinity labeling of the purified cells (see below). After labeling cells were washed once with Hanks’ buffer, pH 7.4, and then transferred into homogenization buffer (10 mM HEPES, 0.1 mM MgCl₂, 1 mM EDTA, 0.34 M sucrose, pH 7.4), and membranes were prepared by N₂ cavitation. To prepare membranes from unstimulated cells, purified neutrophils were directly transferred into homogenization buffer for N₂ cavitation. The low speed supernatant of the cavitated was loaded on a one-step gradient with 7 and 9 ml of 20 and 38% sucrose, respectively, in 10 mM HEPES, pH 7.4, and spun in a Beckman 60Ti rotor for 1 h at 45,000 rpm. The 1.5 ml fractions were collected and the membranes at the 20 and 38% sucrose interface, as judged by alkaline phosphatase activity, were pooled and stored in frozen aliquots at −70°C. The protein concentration was typically 0.2 to 0.4 mg/ml (10⁷ cell equivalents) and contained less than 5% of the total myeloperoxidase activity present in the gradients.

Photoaffinity labeling

Photoaffinity labeling of FPR with 5 to 7 nM [FMLP-LysN⁺[(25)]2[(p-azidosalicylamido)ethyl-1,3'-dithiopropionate (MLFK[(25)]2ASD), formerly called FMLPL>SASD[(25)]2; ref. 32] in plasma membranes was performed as described by Allen et al. (32) with 10 to 20 μg of protein (approximately 10⁸ cell equivalents) in a total volume of 100 μl. Labeling of FPR in cells was conducted before N₂ cavitation with 4 × 10⁷ cells in Hanks’ buffer, pH 7.4, in total volume of 2.5 ml. Neutrophils were incubated with the radioligand for 20 min at 15°C. In some experiments cells were incubated for 5 min at 4°C or 2 min at 37°C. After incubation with the photoreactive peptide the cell suspension was UV irradiated for 10 min on ice as described (32). After labeling cells were diluted into 10 vol of Hanks’ buffer then spun down and resuspended in homogenization buffer.

Solubilization of plasma membranes

Plasma membranes of approximately 10⁷ cell equivalents were solubilized for 1 h on ice in 100 μl solubilization buffer (20 mM HEPES/3 mM MgCl₂, pH 7.4, containing 0.5% TX-100; in some experiments 1% TX-100 or 1% OG was used).

Velocity sedimentation of receptors on sucrose density gradients

The solubilized membranes were loaded onto 5 ml to 20% sucrose density gradients in the respective solubilization buffer. Routinely 0.5% TX-100 was used; however, increasing the TX-100 concentration during solubilization and in the gradients to 1% did not change results. The gradients were spun for 6 or 16 h in a Beckman SW 55Ti rotor at 45,000 rpm. The gradients were fractionated into 400 μl fractions and the pellet material was dissolved in electrophoresis sample buffer. The fractions and pellets were counted in a γ-counter. An aliquot of each fraction was used for SDS-PAGE. The gels were stained with Coomassie blue and then dried. The dried gels were exposed to Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) and the autoradiograms were scanned with a laser densitometer (Biomed Instruments, Inc., Fullerton, CA). Alternatively, the dried gels were exposed to Molecular Dynamics Storage Phosphor Screens and then scanned with a Molecular Dynamics Phosphorimager. The receptor bands were quantitated with the Image Quant software.

For some experiments FPR from TX-100-containing sucrose gradients were partially purified using a WGA-Sepharose affinity matrix (31).

Results

Partial solubilization of FPR by Triton X-100

We have previously suggested that the submembranous cytoskeleton or membrane skeleton may be involved in
OCTYLGLUCOSIDE

**TRITON X-100**

**FIGURE 1.** Velocity sedimentation of solubilized FPR in detergent-containing sucrose density gradients. Receptors were photoaffinity labeled in plasma membranes from unstimulated human neutrophils then solubilized and sedimented into 5 to 20% sucrose density gradients as described in Materials and Methods. The top panel shows that receptors solubilized in OG sedimented to fractions 2 and 3, whereas almost no receptor was found in the pellet (P). However, approximately 50% of the receptors solubilized in TX-100 (lower panel) sedimented to the pellet along with the membrane skeleton. Shown are the autoradiograms of SDS-PAGE gels of the fractions of the sucrose gradients (6 h spins).

Regulation of FPR function (12, 16). To probe the molecular basis for the interaction of receptors with this structure we prepared plasma membranes from unstimulated human neutrophils and extracted them with TX-100 after photoaffinity labeling of the receptors. The extracts were then spun over detergent-containing sucrose density gradients to separate the native receptors by size. It has been reported previously that OG-solubilized FPR from responsive, unstimulated neutrophils sediment as 7S complexes (30). With the shorter centrifugation times used in this study (6 h) 95% of the receptors were found in a peak at fraction 3 (Fig. 1, top). After extraction and sedimentation in TX-100, a detergent that does not disrupt the membrane skeleton at low ionic strength and divalent cation-containing buffer, only 50% of the receptors were found in a peak in fraction 2 (Fig. 1, bottom). However, the remaining 50% of the receptors were recovered in the pellet fraction along with the membrane skeletal actin (Fig. 1, bottom; Fig. 2, left) suggesting that these receptors are somehow physically linked to membrane skeletal structures. OG appears to disrupt this linkage because the actin distribution in the gradients is similar to the distribution when TX-100 is used (not shown). Solubilization in the presence of 600 mM KCl, a condition known to disrupt actin filaments, released virtually all receptors from the pellet (Fig. 2, top). However, washing of the membranes with 600 mM NaCl or KCl before solubilization does not affect the receptor distribution in the gradients suggesting that receptor linkage to the membrane skeletal pellet is not caused by membrane-associated cytosolic actin. The presence of guanyl nucleotides in the solubilization buffer did not change the amount of receptor in the pellet (not shown).

**Disruption of actin filament network increases fraction of solubilized FPR**

SDS-PAGE followed by Western blotting of the individual fractions of sucrose density gradients using antiactin antibodies showed that the KCl-induced release of FPR paralleled the release of actin from the pellet (Fig. 2, bottom). The KCl concentration dependence for receptor release is shown in Figure 3. The radioactivity in the individual fractions of the sucrose density gradients was counted and the percentage of the radioactivity in the pellet was determined for different experiments. The radioactivity remaining in the pellet even at the highest KCl concentrations represents nonspecific labeling of a 68 kDa protein that appears to various degrees in different experiments (8).

pCMPS is a compound that has been shown to solubilize actin from erythrocyte membranes (33). This organic mercurial compound reversibly depolymerizes actin at 0.2 mM, whereas at higher concentration (1 mM) induces irreversible depolymerization. Applied to our system both concentrations released photoaffinity-labeled receptors and actin from the TX-100 insoluble pellet (Fig. 4). Another method to depolymerize actin DNase I was used (34). DNase I present in the solubilization buffer at a concentration of 1 mg/ml released FPR and actin to a similar extent (Table 1). It is of interest that after all these treatments intermediate sedimenting forms of receptor were not detected.

**Desensitization of cells increases insoluble fraction of FPR**

To examine the functional relevance of the observed coupling of FPR to the membrane skeleton, the sedimentation behavior of FPR from unstimulated fully responsive cells was compared with that from fully and partially desensitized neutrophils. Cells were incubated with or without the
photoaffinity ligand at 15°C for 20 min to produce desensitized or unstimulated cells, respectively. This temperature prevents internalization of receptors, inhibits formyl peptide-stimulated superoxide production by 94 ± 6% (n = 3), and slows the actin polymerization response (12, 17). Partially desensitized cells were prepared by incubating neutrophils with ligand at 4°C for 5 min. At this short-end incubation time at lower temperature formyl peptide-stimulated superoxide production is inhibited only by 24 ± 14% (n = 3) indicating a reduced level of desensitization (35, 36). At the end of the respective incubation periods cells were UV-irradiated to photoincorporate the radioligand. Then plasma membranes were prepared from these pretreated cells. The autoradiograms of such an experiment are shown in Figure 5 and reveal that in desensitized cells (15°C) virtually all receptors are shifted to the pellet compared with the partially desensitized cells (4°C) where a significant fraction of receptors remains soluble in TX-100. The TX-100 insoluble receptors from both stimulated and unstimulated cells could be released with KCl in the solubilization buffer with an identical concentration dependence.

FIGURE 3. Concentration dependence of the KCl-induced receptor release from pellets in TX-100-containing sucrose density gradients. The radioactivity of the individual fractions of sucrose density gradients was counted in a γ-counter and the counts in the pellets are expressed as percentage of the control with no KCl. This approach seems to be reasonable because most counts from high m.w. components represent receptors (see Fig. 2). Shown are the mean values from three independent experiments.

FIGURE 2. Distribution of TX-100-solubilized FPR and actin on sucrose density gradients. The autoradiograms in the top panels show that a major portion of the photoaffinity-labeled receptors sediment to the pellet (left), which can be released from the pellet when the solubilization buffer contained 600 mM KCl. The lower panels show the respective actin distribution in Western blots from the same gradients and confirm that KCl treatment depolymerized the sedimentable F-actin almost completely.

FIGURE 5.
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**FIGURE 4.** Receptor and actin release from pellets in TX-100-containing sucrose density gradients with pCMPS. The mercurial compound pCMPS, which depolymerizes actin reversibly or irreversibly at 0.2 and 1 mM, respectively, released receptors from the pellet in TX-100-containing sucrose density gradients and actin to a similar extent. The FPR release has been determined as described in Figure 3 and the actin release as in Table I. The amounts of radioactivity (black bars) and actin (grey bars) in the pellet are given as percentage of control values in the absence of pCMPS and represent the means of three to five independent experiments.

Densitometric scans of autoradiograms of identical experiments as the ones shown in Figure 5 are shown in Figure 6. The values are means of six different experiments from four independent cell preparations. The average percentage of receptors in the pellet in the unstimulated cells was 70.3 ± 3.1% and significantly less than stimulated cells were (93.2 ± 1.4%) of the receptors found in the pellet. Also included are the results from experiments with unstimulated cells where only 50.3 ± 3.5% of the receptors sedimented to the membrane skeletal pellet. Figure 7 demonstrates that the protein composition of unstimulated and desensitized membranes and the respective membrane skeletal pellets was virtually identical as judged by silver-stained SDS-PAGE gels.

It was also tested whether dhCB affects the shift of FPR into the membrane skeleton that parallels desensitization. Neutrophils were preincubated with dhCB (2 μg/ml) for 15 min at 37°C and then incubated with fMLFK-125I-ASD at 4, 15, or 37°C. dhCB was present in all steps including N2 cavitation of the neutrophils. Membranes from labeled cells were prepared as described in Materials and Methods. At all temperatures the receptor distribution in the sucrose gradients was identical in the presence and absence of dhCB.

**Table I. FPR and actin release from the membrane skeleton with DNase I**

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<th>FPR in Pellet (%)</th>
<th>Actin in Pellet (%)</th>
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<tr>
<td>Control</td>
<td>48.2 ± 6.0</td>
<td>35.4 ± 3.9</td>
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<tr>
<td>DNase I</td>
<td>23.2 ± 0.8</td>
<td>17.5 ± 2.4</td>
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Photoaffinity-labeled membranes were extracted in solubilization buffer in the presence and absence of DNase I (1 mg/ml). The extracts were then spun on 5 to 20% sucrose density gradients. The receptor distribution in the gradients was determined by laser densitometric analysis of autoradiograms of dried SDS-PAGE gels of the gradient fractions as described in Materials and Methods. The images of actin Western blots generated with a Micro Computer Imaging Device (Imaging Research Inc, Brock University, Ontario, Canada) were quantitated using the Image Quant software. Values are the means ±SEM from four to six experiments given as percentage of total FPR or actin in the gradient.

**FPR uncoupled from membrane skeleton can still interact with actin**

Figure 2 shows that FPR uncoupled from the membrane skeleton cosediment with actin in sucrose density gradients. Therefore, the question arises whether FPR not associated with the membrane skeleton can interact with actin in the soluble state. To address this question, FPR were partially purified with WGA-Sepharose from actin- and receptor-containing fractions of a sucrose density gradient. The WGA eluates were incubated with antiactin serum or control serum and resedimented in 5 to 20% sucrose density gradients (0.5% TX-100). Figure 8 shows the results of a PhosphorImager analysis of such a gradient. Approximately 18 to 29% of the receptors were found in the pellet after incubation with antiactin antibody in three independent experiments, whereas approximately 2 to 7% of the receptors were pelleted by control serum. These results suggest that FPR may, indeed, directly interact with actin.

**Discussion**

There is a growing body of evidence that the cytoskeleton and/or the membrane skeleton may be implicated in the regulation of various membrane-bound receptors. Many studies showed that receptors are found in the TX-100-insoluble fraction of cells suggesting an association of receptors and the cytoskeleton (19, 21, 22, 37). However, these experiments cannot distinguish between association of receptors with the cytoskeleton or the membrane skeleton. A few studies using partially purified plasma membranes demonstrated association of receptors with the membrane skeleton (23, 38), a distinct cellular structure that has been shown to be different from the three-dimensional cytosolic actin network (25). We have undertaken this study, therefore, to characterize the presumably more selective association of FPR from human neutrophils with the membrane skeleton. Our results support the hypothesis that involves this association with desensitization (12, 18).
FIGURE 5. Sedimentation profile of FPR from partially desensitized (4°C) vs fully desensitized (15°C) neutrophils. Shown are the autoradiograms of SDS-PAGE gels of fractions from a representative set of gradients of the experiments shown in Figure 6.

Contrasts and similarities of cytoskeletal and membrane skeleton preparations

FPR of plasma membranes from human neutrophils solubilized in TX-100 sediment to the pellet in sucrose density gradients along with actin suggesting a linkage of these receptors to the membrane skeleton. This confirms previous results of transient receptor association with the TX-100-insoluble cytoskeleton in human neutrophils (16). Similar findings have also been reported for extracts from whole neutrophils by Särndahl et al. (39). However, several differences are evident when our results using isolated membranes are compared with the latter whole cell studies. Solubilization of membranes in the presence of 600 mM KCl released virtually all receptors from the membrane skeletal pellet along with actin (Fig. 2). In the experiments with cells, only approximately 50% of the receptors could be dissociated from cytoskeletal association (39). In the membrane skeletal preparation we could not induce any release of receptors by preincubating the neutrophils with dhCB. Association of IgE receptors in RBL-243 cells with the membrane skeleton displays a similar insensitivity to treatment of the cells with various cytochalasins (23). Treatment of cells with cytochalasins, however, effectively disrupted receptor-cytoskeleton complexes when intact neutrophils were extracted by detergents (15, 39). dhCB may alter the size of the receptor-bearing cytoskeletal units resulting in inability to cosediment with bulk cytoskeleton (40). Such differences may reflect different coupling of receptors to the cytoskeleton vs the membrane skeleton, which are comprised of structurally different actin networks (23, 25, 28). Other differences in the experimental approaches might also be responsible for the contrasting results.

Neutrophils modulate FPR expression at the cell surface by mobilization of intracellular pools (41, 42) or by internalization. Such processes might interfere with interpretation of TX-100 insolubility of FPR in whole cells (39). In the experiments described here, cells were stimulated at 15°C to inhibit these mobilization and internalization processes without perturbing desensitization. The plasma membranes were isolated, thus allowing us to observe the events taking place in the membranes independent of these processes. Although our results suggest that the interaction of FPR with the membrane skeleton involves actin polymers, it appears that this interaction is not sensitive to dhCB treatment as opposed to other actin-mediated events, including slowing the formation of a high affinity FPR complex (12, 17). Indeed, dhCB also does not appear to alter the agonist-induced receptor redistribution in the plasma membrane of neutrophils that were also incubated at 14°C to inhibit receptor internalization (43). Botulinum C2 toxin, which ADP ribosylates nonmuscle G actin, has also been used to probe the role of actin in neutrophil activation and has been shown to be similar to dhCB in its effects on superoxide production and secretion at 37°C (44). In this case the binding characteristics of the receptor were virtually unchanged by botulinum C2 toxin (45),...
DISTRIBUTION OF SOLUBILIZED FPR ON SUCROSE DENSITY GRADIENTS

<table>
<thead>
<tr>
<th>Fraction 1-5</th>
<th>Pellet</th>
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<tr>
<td>M</td>
<td>100</td>
</tr>
<tr>
<td>4°C</td>
<td>97</td>
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<tr>
<td>15°C</td>
<td>91</td>
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FIGURE 6. Distribution of FPR from unstimulated vs desensitized neutrophils on sucrose density gradients. FPR were photoaffinity labeled in membranes prepared from unstimulated neutrophils (M). To desensitize the cells FPR were labeled directly in neutrophils at 15°C. Neutrophils were also labeled at 4°C, a condition that partially inhibits desensitization. The isolated plasma membranes of these cells were then solubilized in TX-100 and sedimented in sucrose density gradients. Approximately 50% of the FPR from unstimulated cells sedimented to the pellet and the pelleting portion of the receptors from cells labeled at 4°C amounted to approximately 70%. Virtually all (>90%) receptors from fully desensitized neutrophils (15°C) sedimented to the pellet. Shown are the results of densitometric scans of autoradiograms (means ± SEM of six experiments).

whereas dhCB inhibited the formation of the slowly dissociating state characteristic for cytoskeleton-associated receptors (17). These studies, however, do not address the existence of various actin pools in the cell (46), which are functionally distinct and may be differentially susceptible to toxins like dhCB or botulinum toxin. The membrane skeleton probably represents one of these actin pools (47) and our experiments were designed to study the interaction of the FPR only with this pool. Thus, the nature of the apparent differences observed relative to these inhibitors may involve their relative effects on these different pools.

FPR association with the membrane skeleton and desensitization

In cells desensitized at 15°C with photoaffinity ligand, virtually all receptors are shifted to the membrane skeletal pellet (Figs. 5 and 6). The reduced coupling observed at 4°C might be considered of minor importance because 70% of the receptors are still found associated with the membrane skeleton. However, previous studies (35, 36) have shown that only partial inhibition of desensitization

FIGURE 7. Protein composition of membranes and membrane skeletal pellets from unstimulated and desensitized neutrophils. Shown are silver-stained SDS-PAGE gels of membranes and membrane skeletal pellets from unstimulated (U) and desensitized (D) neutrophils. The membrane skeletal pellets were prepared in sucrose density gradients as described in Materials and Methods. An equivalent amount of membranes from the respective cells was directly dissolved in electrophoresis sample buffer and was analyzed in parallel.

FIGURE 8. Immunosedimentation of FPR with antiactin antibodies. The top fractions of a sucrose density gradient (16 h spin) containing actin and receptors (compare Fig. 2) were incubated with antiactin antibodies (filled columns) or control serum (open columns) and resedimented in a detergent-containing sucrose gradient for 16 h. Approximately 25% of the receptors sediment to the pellet in the presence of antiactin antibodies, whereas only 5% of the receptors were found in the pellet in the presence of control serum. The numbers on the x-axis refer to fraction numbers; 13, pellet.
is achieved at this temperature. It is highly significant, therefore, that FPR labeled in membranes from unstimulated neutrophils show only 50% coupling to the membrane skeleton. Because neutrophils are quite sensitive to handling, the possibility that a certain degree of stimulation and desensitization and hence coupling occurred before and during the preparation of the cells cannot be ruled out. Another possibility could be that the photoaffinity probe converts receptors into the membrane skeleton-bound form during labeling of the membranes. However, labeling of FPR in plasma membranes from unstimulated cells at 15 or 37°C does not change the receptor distribution in the sucrose gradients compared with labeling at 4°C suggesting that the photoaffinity probe can convert FPR to the membrane skeleton-bound form only in intact cells (not shown). It is possible that in the basal responsive state of the cell a certain fraction of receptors remains linked to the membrane skeletal matrix. Independent support for this hypothesis can be found in FPR lateral mobility studies which show that approximately 40% of the fluorescent antagonist occupied FPR are immobile (43).

Desensitization of FPR in neutrophils and HL60 cells results in a decreased coupling to G proteins (48, 49). Our current hypothesis about desensitization of FPR views the physical segregation of the receptors from their signal transduction partners, the G proteins, as an important mechanism for turning off the receptor-mediated responses (12). One could speculate that uncoupling of receptors from a G protein would facilitate the interaction with the membrane skeleton. This model seems to be insufficient because it would imply that guanylnucleotides should increase the association of receptors with the membrane skeleton. However, we could not detect any significant effect of guanylnucleotides on the interaction of receptors and membrane skeleton. In contrast, Sämdahl et al. (39) found a release of FPR from cytoskeletal association with 1 mM GTP*PS. However, their experimental protocol cannot rule out a shift of receptors to a low affinity state for agonists by the presence of a guanylnucleotide (50, 51). Therefore, this release might also represent attenuated binding of radiolabeled agonist to the low affinity state of the receptor compared with high affinity binding in control cells rather than release of receptors from the cytoskeleton. This interpretation would also resolve the apparent discrepancy between pertussis toxin treatment and guanylnucleotide treatment in their study. Both treatments result in uncoupling of receptor and G protein and one would expect, therefore, a similar effect on receptor-cytoskeleton interactions.

Role for actin in FPR coupling to the membrane skeleton

Membrane-bound actin appears to play an important role in the coupling of FPR to the membrane skeleton because it can be released if membranes are solubilized under conditions known to disrupt actin polymers. The presence of 600 mM KCl, the organic mercurial compound pCMPS that has been shown to solubilize actin from erythrocyte membranes (33), or DNase I, which can depolymerize a stoichiometric amount of actin (34), induced receptor and actin release from the membrane skeletal pellet in sucrose density gradients to a similar extent. It is not clear from these results whether or not the receptors interact directly with actin. However, the immunosedimentation data shown in Figure 8 suggest that FPR can indeed directly interact with actin. This result shows that at least part of the FPR that are not linked to the membrane skeleton in this protocol can bind to endogenous actin present in the incubation mix. This observation may suggest a direct link between actin and receptors, but it does not exclude the possibility that other proteins are involved in an actin-receptor linkage. In another study, we have investigated this interaction in more detail and these data support direct FPR interaction with actin (52).

Summary and conclusion

The use of purified plasma membranes to study the interaction of FPR with cytoskeletal elements suggests that the membrane skeleton is a relevant cellular structure that is implicated in the regulation of receptor function. In unstimulated cells we find approximately 50% of the receptors associated with the TX-100-insoluble fraction of the membranes. Stimulations of the neutrophils before the isolation of membranes shifts virtually all receptors to this membrane skeleton-associated state. Although we do not have evidence for a direct coupling of FPR to actin filaments we believe that membrane-bound actin, which might be in a different state than the cytosolic actin, plays an important role in the control of FPR function. It is tempting to speculate that the observed interaction of FPR with the membrane skeleton is the basis for the recently described receptor class desensitization, which has only been observed for chemotactic receptors but not for other G-protein-coupled receptors (14).

References

production


