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SHORT COMMUNICATIONS

Physical coupling of *N*-formyl peptide chemoattractant receptors to G protein is unaffected by desensitization

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Abstract—Desensitization of *N*-formyl peptide chemoattractant receptors (FPR) in human neutrophils results in association of these receptors to the membrane skeleton. This is thought to be the critical event in the lateral segregation of receptors and guanyl nucleotide-binding proteins (G proteins) within the plane of the plasma membrane resulting in an interruption of the signaling cascade. In this study we probed the interaction of FPR with G protein in human neutrophils that were desensitized to various degrees. Human neutrophils were desensitized using the photoreactive agonist *N*-formyl-met-leu-phe-lys-N$^{\epsilon}$-[^{125}I]2(*p*-azidosalicylamido)ethyl-1,3′-dithiopropionate (*f*MLFK-[^{125}I]ASD). The interaction of FPR with G protein was studied via a reconstitution assay and subsequent analysis of FPR–G protein complexes in sucrose density gradients. FPR–G protein complexes were reconstituted with solubilized FPR from partially and fully desensitized neutrophils with increasing concentrations of G_i purified from bovine brain. The respective EC_{50} values for reconstitution were similar to that determined for FPR from unstimulated neutrophils (Bommakanti RK *et al.*, *J Biol Chem* **267**: 7576–7581, 1992). We conclude, therefore, that the affinity of the interaction of FPR with G protein is not affected by desensitization, consistent with the model of lateral segregation of FPR and G protein as a mechanism of desensitization.

Key words: chemotactic receptors; G proteins; *N*-formyl peptides; signal transduction; receptor-G protein coupling

FPR† on human neutrophils transduce signals via pertussis toxin-sensitive guanyl nucleotide-binding proteins (G proteins) which activate phospholipase C [1, 2]. Analagous to other members of the family of G protein-coupled receptors, FPR exhibit the phenomenon of desensitization upon prolonged exposure to agonists [3]. Several mechanisms of desensitization have been proposed (reviewed in Refs. 4, 5). It has been shown that receptor phosphorylation of β-adrenergic receptors plays a crucial role in rapid desensitization. This covalent modification of the receptor protein triggers binding of arrestin-like proteins [6] that ultimately leads to uncoupling of receptors from their specific G protein. Although phosphorylation of FPR was demonstrated recently [7, 8] it is not clear whether a similar pathway involving a specific receptor kinase and an arrestin-like protein are involved in FPR desensitization.

For FPR a different mechanism of rapid desensitization appears to be operative. FPR in desensitized neutrophils are shifted to an actin- and fodrin-rich plasma membrane domain that is depleted of G protein [9]. Simultaneously, FPR are found complexed to the membrane skeletal actin as desensitization occurs [10, 11]. We believe that immobilization by coupling to the membrane skeleton is a mechanism to segregate receptors from G protein in the plane of the plasma membrane, thus allowing for physical interruption of the signaling cascade [12]. In agreement with this model are reports showing a decreased coupling of FPR and G protein [13, 14]. It is not yet clear what molecular events determine the coupling of FPR to G protein or the membrane skeleton in responsive and desensitized neutrophils, respectively. With a recently developed reconstitution assay for FPR–G protein complexes that allows us to study the physical interaction of receptor and G protein [15], we investigated whether binding of FPR to G protein was altered during desensitization. Our results show that the apparent affinity of FPR for G protein in desensitized neutrophils was identical to that in responsive cells, suggesting that a modification of FPR causing an altered coupling to the G protein does not occur.

Materials and Methods

Materials. Bovine brain G_i was purified according to Sternweis and Robishaw [16] as described by Bommakanti *et al.* [15]. GTPγS was from Boehringer Mannheim (Indianapolis, IN, U.S.A.) and OG was from Calbiochem (La Jolla, CA, U.S.A.). All other materials were from sources described previously [9, 15, 17]. Procedures for SDS–PAGE have been carried out as described [9].

Cells and membranes. Human neutrophils were prepared as described recently [18]. The cells were desensitized to various degrees by incubation with an agonist at different temperatures. Neutrophils were incubated with the photoaffinity ligand *f*MLFK-[^{125}I]ASD for 5 min at 4° which results in partial desensitization while incubation for 20 min at 15° or for 5 min at 37° causes complete desensitization of the cells [3, 11]. At the end of the respective incubation periods the cell suspensions were UV-irradiated in order to photoincorporate the ligand into the receptor protein. Further details and the preparation of membranes by N_2 cavitation have been described recently [11].

Solubilization of FPR and reconstitution of 7S FPR–G protein complexes. Endogenous FPR–G protein complexes were dissociated by treatment of the membranes with GTPγS with subsequent washing as reported by Bommakanti and co-workers [15, 19]. Plasma membranes of about 7×10^7 cell equivalents were solubilized on ice in 200 μL solubilization buffer (20 mM Hepes/3 mM $MgCl_2$ pH 7.4 containing 1% OG) for 1 hr. Aliquots of these membrane extracts were incubated with increasing concentrations of purified G protein for 6 hr at 4°. Then the incubation mixture was loaded on 5–20% sucrose

† Abbreviations: FPR, *N*-formyl peptide chemoattractant receptors; *f*MLFK-[^{125}I]ASD, *N*-formyl-met-leu-phe-lys-N$^{\epsilon}$-[^{125}I]2(*p*-azidosalicylamido)ethyl-1,3′-dithiopropionate; OG, *n*-octyl-β-D-glucopyranoside.

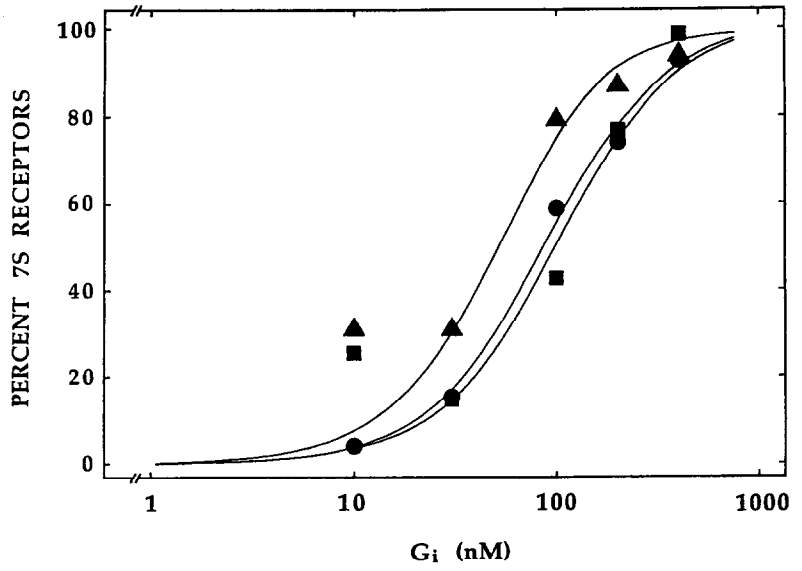

Fig. 1. Reconstitution of 7S FPR–G protein complexes. 7S receptor complexes were reconstituted with FPR solubilized from partially (●) and fully (■) desensitized neutrophils and bovine brain G_i. A control curve with FPR from unstimulated neutrophils is also shown (▲). Partial desensitization was achieved by incubation of neutrophils with *f*MLFK-[^{125}I]ASD for 5 min at 4°. For full desensitization cells were incubated with the photoaffinity ligand for 20 min at 15°. (For experimental details see Refs. 11, 19). For reconstitution of 7S receptor complexes an EC_{50} value of 56 nM was calculated for FPR from responsive neutrophils and 85 and 100 nM for FPR from partially and fully desensitized neutrophils, respectively.

Table 1. EC_{50} values for reconstitution of 7S receptor complexes from solubilized 4S FPR with G protein

State of neutrophil	FPR reconstituted with	EC_{50} (nM)
Responsive	G_i	170* (6)
Responsive	G_n	70* (3)
Partially desensitized, 4°	G_i	98 (2)
Fully desensitized, 15°	G_i	85 (2)
Fully desensitized, 37°	G_i	130 (1)

FPR were photoaffinity labeled in membranes from unstimulated (responsive) neutrophils or after incubation of neutrophils with the photoaffinity ligand at different temperatures which allows desensitization to various degrees (see Materials and Methods [11, 15]). Mean values with the number of experiments given in parentheses are shown. * Data from [15].

density gradients and spun for 8 hr. The gradients were fractionated and the fractions analysed by SDS–PAGE and phosphor imager analysis. For further details see Refs. 15, 19.

Results and Discussion

We used a recently developed reconstitution assay [15] in order to study the interaction of FPR from neutrophils that were desensitized to various degrees with G proteins. Reconstitution of FPR–G protein complexes with G_i was carried out with solubilized receptors from partially desensitized neutrophils (cells photoaffinity labeled at 4°) or fully desensitized neutrophils (cells photoaffinity labeled at 15°) [9, 11, 20]. FPR complexed with G protein sediment as 7S complexes in OG-containing sucrose density gradients that are disrupted to 4S complexes by GTPγS [17]. 7S receptor complexes were reconstituted from 4S receptors with increasing concentrations of bovine brain G_i and complex formation was monitored with sucrose density gradient centrifugation. For reconstitution with FPR from partially desensitized and fully desensitized neutrophils EC_{50} values of 85 and 100 nM, respectively, were determined (Fig. 1, Table 1). Previous studies of the reconstitution of 7S FPR–G protein complexes with FPR from unstimulated responsive neutrophils [15] gave EC_{50} values of 170 nM for G_i and 70 nM for G_n (G protein purified from human neutrophils which is identical to G_{i2} (Table 1). Thus, the affinity of the interaction of receptor and G protein appears to be unchanged by desensitization. We also photoaffinity labeled neutrophils at 37°, a temperature not normally used

for desensitization experiments, because it allows for internalization of FPR [3]. Again, reconstitution occurred with a similar EC_{50} value of 130 nM (Table 1).

Our data suggest that upon desensitization of FPR in human neutrophils these receptors are still fully capable of interaction with G protein. This result is in keeping with the proposal that physical segregation of FPR from G protein within the plane of the plasma membrane serves as the basis for desensitization. A recent lateral mobility study [21] provides support for this segregation which is thought to be achieved by the coupling of FPR to the membrane skeleton. This coupling results in a receptor distribution that is distinct from the distribution of G proteins and thus causes an interruption of the signaling cascade [12, 17]. The coupling of FPR to the membrane skeleton may be mediated by direct binding of FPR to actin [10]. The molecular mechanism of this interaction is unknown but a covalent modification of FPR in analogy with phosphorylation of β-adrenergic receptors that allows for β-arrestin binding [22, 23] cannot be excluded. The recent evidence for phosphorylation of FPR [7, 8] might provide the basis for such a mechanism. However, according to the data of this study, such a modification might only affect the interaction of FPR with actin (and/or other protein(s) involved in desensitization) but leave the FPR–G protein interaction unchanged. Although there is strong evidence for the lateral segregation model of FPR desensitization, additional mechanisms may be operative. The recent evidence for phosphorylation of FPR [7, 8] suggests that these receptors might indeed be uncoupled from G protein by this modification and binding of an arrestin-like protein as has been documented for β-adrenergic receptors and rhodopsin [4, 6].

In conclusion, we have shown that the physical interaction of FPR with G protein is not affected by desensitization of this receptor system in human neutrophils. A recently introduced reconstitution assay that allows for an estimation of the affinity of receptor–G protein interaction [15] gave similar EC_{50} values for this interaction for receptors from unstimulated [15], partially and fully desensitized neutrophils (Fig. 1, Table 1). This result is consistent with the model of lateral segregation of FPR and G proteins into different plasma membrane domains as a mechanism of desensitization [3, 12].

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