Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 10542–10546, November 1992
Physiology

High- and low-affinity binding of GROα and neutrophil-activating peptide 2 to interleukin 8 receptors on human neutrophils

(cross-linking/solubilization/binding studies/guanine nucleotide binding protein)

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Communicated by Ewald R. Weibel, July 9, 1992

ABSTRACT **GROα and neutrophil-activating peptide 2 (NAP-2), like their analog interleukin 8 (IL-8), are considered to be inflammatory mediators since they recruit and activate neutrophil leukocytes. After introduction of tyrosines by substitution for other residues at the C terminus, GROα and NAP-2 were labeled with ^{125}I and used for binding studies. A total of 60,000–90,000 receptors per neutrophil were found for either ligand. Of these 30–45% were of high affinity with a mean K_d value of 0.3 and 0.7 nM for GROα and NAP-2, respectively, and 55–70% of low affinity (K_d = 30 nM). Two proteins of ≈70 kDa and 44 kDa (p70 and p44) were specifically cross-linked with labeled GROα, NAP-2, and IL-8. Unlabeled IL-8 fully inhibited this cross-linking and the binding of labeled GROα or NAP-2 to the high-affinity sites on neutrophils or neutrophil membranes. Treatment of membranes with digitonin resulted in the preferential solubilization of a single receptor species, corresponding to p44, that bound GROα and NAP-2 with low affinity (K_d = 30 nM) and IL-8 with high affinity (K_d = 0.4 nM). Exposure of neutrophil membranes to 100 μM guanosine 5′-[γ-thio]triphosphate led to a 75-fold increase of the K_d in ≈60% of the IL-8 receptors. High-affinity receptors for GROα and NAP-2 were similarly affected. In contrast, guanosine 5′-[γ-thio]triphosphate had no effect on the binding of IL-8 to p44 solubilized by digitonin. These results demonstrate that human neutrophils bear two classes of receptors for GROα, NAP-2, and IL-8 (p70 and p44) that may differ in their mode of interaction with GTP regulatory proteins.**

Among the growing number of interleukin 8 (IL-8)-related chemotactic cytokines, neutrophil-activating peptide 2 (NAP-2) and GROα were studied extensively because of their possible involvement in the pathophysiology of inflammation (1–4) and tumor growth (5, 6). Responses with neutrophils, the principal target cells for all three cytokines, include chemotaxis, shape change, mobilization of cytosolic free calcium, release of granule components, upregulation of adhesion receptors, and the respiratory burst (7–12).

Several recent reports have described receptors for IL-8 on human neutrophils (13–17). With one exception (13), these studies show that IL-8 binds to a single class of high-affinity receptors (K_d = 0.2–4 nM) with densities reported to be between 20,000 and 75,000 sites per cell. Cross-linking experiments revealed either one (14) or two (15, 17) receptor proteins with molecular masses ranging from 44 to 78 kDa. Recently, cDNAs for two IL-8 receptors with seven putative transmembrane domains typical of guanine nucleotide binding protein (G protein)-coupled receptors have been described (18, 19).

Studies of the biochemical and binding properties of receptors for GROα and NAP-2 were hampered by the absence of tyrosines suitable for radioiodination. By substitution of residues at or close to the C terminus with tyrosines, we have obtained analogs with similar biological activities as the natural peptides that could be labeled to high-specific activities with ^{125}I. Using these analogs, we were able to identify GROα and NAP-2 receptors on human neutrophils by direct binding assays and to compare them with the receptors for IL-8. The results of the present paper demonstrate the existence of two distinct receptors on human neutrophils that recognize GROα and NAP-2 as well as IL-8.

MATERIALS AND METHODS

Materials. Aprotinin, chymostatin, leupeptin, bovine serum albumin, EDTA, PEG 6000, diisopropyl fluorophosphate, deoxycholate, Nonidet P-40, polyethylenimine, and D-(+)-sucrose were obtained from Fluka; gelatin and phenylmethylsulfonyl fluoride were from Sigma; disuccinimidyl suberate was from Pierce; DNase I was from Boehringer Mannheim; 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and dodecyl β-maltoside were from Calbiochem–Behring; digitonin and Hepes were from Merck; RPMI 1640 medium was from GIBCO/BRL; Na[^{125}I] was from Amersham; and prestained SDS/PAGE molecular weight markers were from Bio-Rad.

Preparation and Solubilization of Membranes. Human neutrophils were isolated from buffy coats of donor blood (8). The final suspension of 2×10^8 cells per ml in 0.15 M NaCl/0.05 mM $CaCl_2$ consisted of 95–98% granulocytes and was kept at 10°C until use. For the preparation of membranes, neutrophils at 2×10^8 cells per ml were disrupted by nitrogen cavitation at 30 bar (1 bar = 100 kPa) for 20 min at 4°C in phosphate-buffered saline supplemented with 2.5 mM $MgCl_2$, 0.1% gelatin, DNase I (100 units/ml), aprotinin (10 μg/ml), chymostatin (10 μg/ml), and leupeptin (10 μg/ml), and 0.1 mM phenylmethylsulfonyl fluoride. The cavitated suspension was mixed with 1 vol of 0.5 M $KHCO_3$/25 mM EDTA/protease inhilabitors (as above) and subjected to differential centrifugation in three steps at 4°C, 400 × *g* for 20 min, 10,000 × *g* for 10 min, and 100,000 × *g* for 45 min. Membranes in the 100,000 × *g* pellet were resuspended in 0.25 M sucrose buffered with 10 mM Hepes (pH 7.2) containing the protease inhibitors and stored in aliquots (0.8–1.5 mg of protein per ml) at −70°C.

Digitonin-solubilized membrane proteins were prepared essentially as described by Rollins *et al.* (20). One volume of membrane suspension was mixed with an equal volume of 2% (wt/vol) digitonin in 50 mM Hepes, pH 7.2/protease inhibitors. The mixture was incubated for 1 h on ice and centrifuged at 200,000 × *g* for 10 min (Beckman TL-100 centrifuge),

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Abbreviations: NAP-2, neutrophil-activating peptide 2; IL-8, interleukin 8; GTP[γS], guanosine 5′-[γ-thio]triphosphate; G protein, guanine nucleotide binding protein.

and the supernatants (0.5–0.9 mg of protein per ml) were kept on ice for subsequent use or stored at −70°C.

Synthesis and Binding of GROα(Y) and NAP-2(Y). IL-8, GROα, GROα(Y), NAP-2, and NAP-2(Y) were prepared by stepwise chemical synthesis on an Applied Biosystems model 430A peptide synthesizer using double-couple protocols essentially as described (12). GROα(Y) was obtained by the replacement of Asn-73 with Tyr at the C terminus, and NAP-2(Y) was obtained by the replacement of Ser-68 and Asp-70 with Tyr. Routinely, 1 nmol of peptide was iodinated with Enzymobead reagent (Bio-Rad), characterized (0.9 to 2 × 10^{18} dpm/mol of peptide ligand), and used in whole-cell binding assays exactly as described (17). Neutrophil membranes (8–10 μg of protein) or digitonin-solubilized membrane proteins (15–20 μg of protein) were incubated at 21°C for 90 min in a total volume of 120 μl with 0.2–200 nM iodinated IL-8, GROα(Y), or NAP-2(Y) in the presence or absence of unlabeled ligands. Membrane-bound radioactivity was retained on Whatman GF/C filters pretreated with 0.33% polyethylenimine using a Millipore filtration apparatus model 1225. In experiments with solubilized receptors, the incubation samples were mixed with 50 μl of 40% (wt/vol) PEG 6000 and placed on ice for 20 min prior to filtration, to precipitate the proteins. The filters were washed three times with ice-cold phosphate-buffered saline containing 0.1% bovine serum albumin (membranes) or 9% PEG 6000 (solubilized receptors), and dried, and radioactivity was measured.

The number of binding sites (60,000–90,000 receptors per cell; 2–7 × 10^9 receptors per μg of membrane protein; 0.7–1.4 × 10^9 receptors per μg of solubilized protein), the dissociation constants, and the nonspecific binding parameters were determined by computer modeling as described (17). Nonspecific binding did not exceed 3% (with whole cells), 5% (with membranes), and 11% (with solubilized proteins) of the respective free ligand concentrations.

Cross-Linking Experiments. The protocol for labeling of neutrophil receptors on whole cells with iodinated IL-8 has been described in detail (17) and was applied in cross-linking experiments with iodinated GROα(Y) and NAP-2(Y). For cross-linking studies with soluble receptors, membranes were freshly solubilized as described above and 60–100 μg of soluble proteins was incubated in a total volume of 370 μl with 0.3–4 nM of iodinated IL-8, GROα(Y), or NAP-2(Y) in the presence or absence of unlabeled ligands at 21°C for 90 min. After cross-linking with 1 mM disuccinimidyl suberate for 15 min at 21°C, 40 μl of 1 M Tris·HCl (pH 7.4) was added and the soluble proteins were sedimented by incubation with 140 μl of 40% PEG 6000 for 5 min on ice and centrifugation at 15,000 × *g* for 10 min. The proteins in the pellets were analyzed by SDS/PAGE and autoradiography as described above.

Elastase Release Assay and Protein Determination. The biological activity of GROα, NAP-2, and analogs was assessed by measuring the release of elastase from human neutrophils pretreated with cytochalasin B (8, 12). Protein was determined using the kit Micro BCA assay (Pierce).

RESULTS

Tyrosine-Substituted Ligands. The tyrosine-substituted peptides GROα(Y) and NAP-2(Y) were compared with natural GROα and NAP-2 for activation of human neutrophils and binding to cellular receptors. As shown in Fig. 1*A*, GROα and GROα(Y) were equally active in induction of elastase release, whereas NAP-2(Y) was slightly more potent than NAP-2. Both, the natural and modified cytokines competed with the same efficiency with their iodinated counterparts for binding to neutrophils (Fig. 1*B*). In agreement with our former observations (17), GROα, NAP-2, and the tyrosine-substituted derivatives did not displace ^{125}I-labeled IL-8 as efficiently as unlabeled IL-8. In addition, in contrast to displacement with unlabeled IL-8, the competition curves obtained with unlabeled GROα(Y), NAP-2(Y), and their natural forms were not sigmoidal (Fig. 1*C*).

Binding to Neutrophils. Since incorporation of tyrosine residues did not substantially affect competition for IL-8 binding or biological activity, radioiodinated GROα(Y) and NAP-2(Y) were used for direct binding studies. Human neutrophils were incubated for 90 min at 4°C with increasing concentrations of ^{125}I-labeled GROα(Y) or ^{125}I-labeled NAP-2(Y) in the presence or absence of an excess of unlabeled ligand, and the binding data were analyzed. Best fitting as assessed by the least values of the sum of squares of residuals was achieved by applying a two-binding-site model. Fig. 2 shows Scatchard plots of representative binding experiments. Analysis of independent experiments revealed that for both ligands 30–45% of the total binding sites were of high affinity [K_d = 0.3 ± 0.1 nM for GROα and 0.7 ± 0.1 nM for NAP-2 (mean ± SEM)], whereas the remaining binding sites were of low affinity (K_d = 30 ± 5 nM). The calculated number

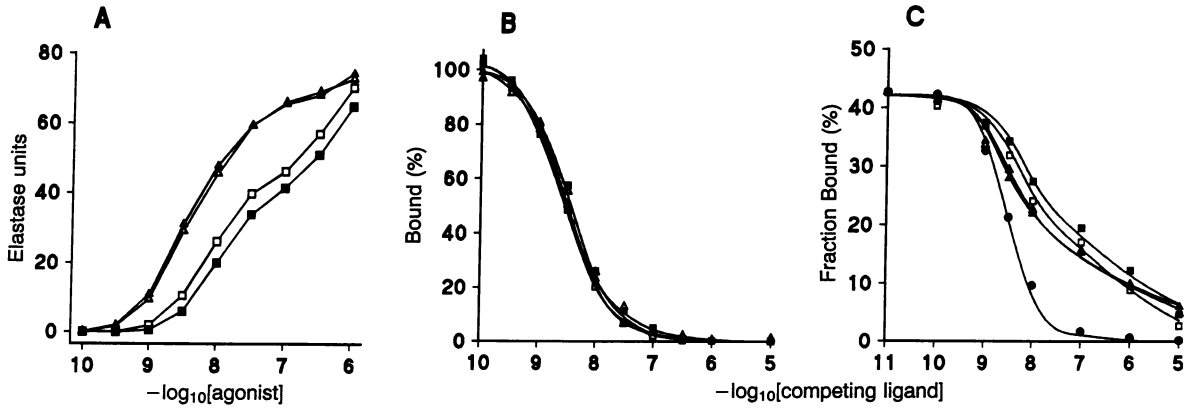

FIG. 1. Comparison of GROα(Y) and NAP-2(Y) with their natural forms. (*A*) Release of elastase from cytochalasin B-treated human neutrophils after stimulation with GROα(Y) (△), GROα (▲), NAP-2(Y) (□), or NAP-2 (■). One unit corresponds to 1 pmol of 7-amino-4-methylcoumarin produced per min by 10^6 cells. (*B*) Inhibition of ^{125}I-labeled GROα(Y) and ^{125}I-labeled NAP-2(Y) binding by their natural and modified analogs. Approximately 2 × 10^6 neutrophils were incubated with 2 nM of either iodinated ligand for 90 min at 0–4°C in the presence of increasing concentrations of unlabeled GROα(Y) (△) or GROα (▲) for ^{125}I-labeled GROα(Y) and unlabeled NAP-2(Y) (□) or NAP-2 (■) for ^{125}I-labeled NAP-2(Y). (*C*) Competition for IL-8 binding. Neutrophils were incubated as above with 1 nM ^{125}I-labeled IL-8 in the presence of increasing concentrations of unlabeled IL-8 (●), GROα(Y) (△), GROα (▲), NAP-2(Y) (□), or NAP-2 (■). Fraction bound (%) corresponds to the fraction of receptors occupied by ^{125}I-labeled IL-8.

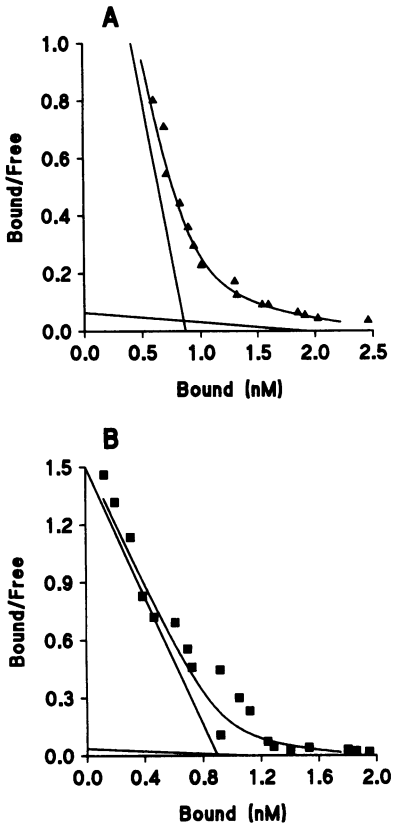

FIG. 2. Steady-state binding of ^{125}I-labeled GROα(Y) and ^{125}I-labeled NAP-2(Y) to human neutrophils. Cells were incubated as described in Fig. 1*B* with ^{125}I-labeled GROα(Y) (*A*) or ^{125}I-labeled NAP-2(Y) (*B*) in the presence or absence of a 100- to 800-fold excess of the respective unlabeled ligand. The data are presented as Scatchard plots (1 nM bound corresponds to 60 fmol of ligand bound to 10^6 cells and 1 unit of bound/free corresponds to 60 μl per 10^6 cells).

of receptors (60,000–90,000 sites per neutrophil) was similar for GROα and NAP-2. In competition experiments increasing concentrations of unlabeled IL-8 led to progressive and complete inhibition of the binding of ^{125}I-labeled GROα(Y) or ^{125}I-labeled NAP-2(Y) (data not shown). The potency of IL-8

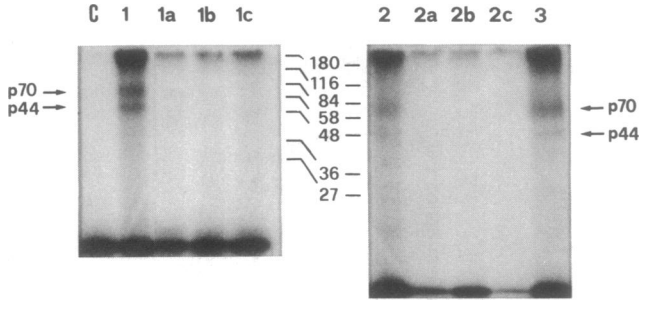

FIG. 3. Chemical cross-linking of ^{125}I-labeled GROα(Y), ^{125}I-labeled NAP-2(Y), and ^{125}I-labeled IL-8 to human neutrophils. Approximately 6×10^6 cells were incubated in 120 μl for 90 min at 4°C with 3 nM ^{125}I-labeled NAP-2(Y) (lanes 1–1c), ^{125}I-labeled GROα(Y) (lanes 2–2c), or ^{125}I-labeled IL-8 (lane 3) either alone or in the presence of a 500-fold excess of unlabeled IL-8 (lanes 1a and 2a), NAP-2(Y) (lanes 1b and 2b), or GROα(Y) (lanes 1c and 2c). After cross-linking, membranes were prepared and the labeled membrane proteins were analyzed by SDS/PAGE and autoradiography. As a control (lane C), cells were incubated with ^{125}I-labeled NAP-2(Y) in the absence of cross-linking agent. Molecular mass values for the protein standards are indicated in kDa. Arrows indicate the cross-linked products (p70 and p44; molecular masses for the ligands were subtracted).

to compete with ^{125}I-labeled GROα(Y) or ^{125}I-labeled NAP-2(Y) for binding was similar as the displacement of ^{125}I-labeled IL-8 with unlabeled IL-8 (Fig. 1*C*), indicating that the high-affinity receptors for GROα and NAP-2 also recognized IL-8 with high affinity. High-affinity receptors for GROα and NAP-2 that did not bind IL-8 were not detected.

Fig. 3 shows autoradiograms from cross-linking experiments after incubation of neutrophils with either ^{125}I-labeled GROα(Y) or ^{125}I-labeled NAP-2(Y). Two protein bands of $\approx$70 kDa (p70) and 44 kDa (p44) were specifically labeled with both ligands as well as ^{125}I-labeled IL-8. The cross-linking with ^{125}I-labeled GROα(Y) and ^{125}I-labeled NAP-2(Y) was prevented by a 500-fold excess of unlabeled GROα, NAP-2, or IL-8.

Binding to Cell-Free Preparations. Membranes from human neutrophils prepared by nitrogen cavitation and differential centrifugation retained the capacity to bind iodinated GROα(Y), NAP-2(Y), and IL-8. The binding properties for the various ligands were not altered: A single high-affinity class of receptors was found for IL-8, whereas GROα and NAP-2 bound to high- and low-affinity sites as in intact cells (data not shown).

Among several detergents that were tested for solubilization of neutrophil receptors, digitonin was found to be most satisfactory. Scatchard evaluation of equilibrium binding data (Fig. 4*A*) revealed a slight decrease of the binding affinity

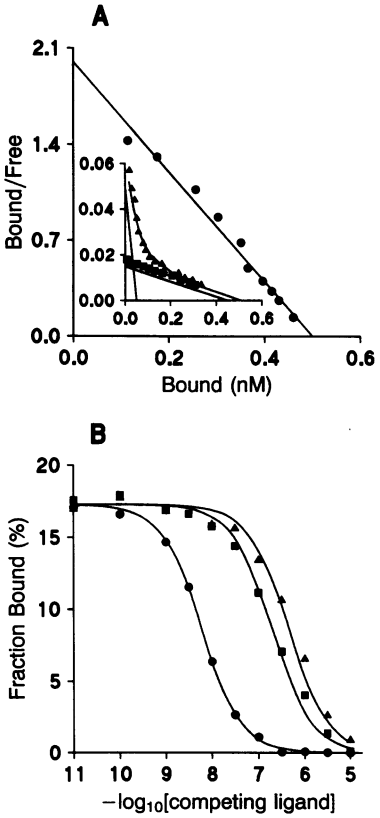

FIG. 4. Steady-state binding of ^{125}I-labeled GROα(Y), ^{125}I-labeled NAP-2(Y), and ^{125}I-labeled IL-8 to soluble receptors. (*A*) ^{125}I-labeled GROα(Y) (▲), ^{125}I-labeled NAP-2(Y) (■), or ^{125}I-labeled IL-8 (●) (0.5–120 nM) was incubated with 15 μg of solubilized membrane protein in the presence or absence of a 100- to 1500-fold excess of unlabeled GROα(Y), NAP-2(Y), or IL-8 (1 nM bound corresponds to 8 fmol of bound per μg protein and 1 unit of bound/free corresponds to 120 μl/15 μg of protein). (*B*) Competition of binding of 2 nM ^{125}I-labeled IL-8 to 33 μg of solubilized membrane protein with increasing concentrations of unlabeled IL-8 (●), GROα(Y) (▲), or NAP-2(Y) (■). Samples were processed as in *A*. Fraction bound (%) was as in Fig. 1*B*.

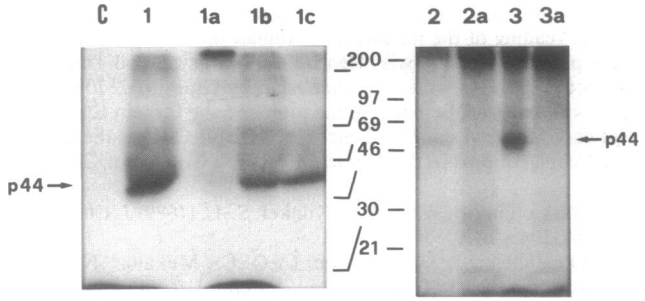

FIG. 5. Chemical cross-linking of ^{125}I-labeled IL-8, ^{125}I-labeled GROα(Y), or ^{125}I-labeled NAP-2(Y) to soluble receptors. Solubilized membrane protein (100 μg) was incubated for 60 min at 21°C with 1 nM ^{125}I-labeled IL-8 (lanes 1–1c), ^{125}I-labeled GROα(Y) (lanes 2 and 2a), or ^{125}I-labeled NAP-2(Y) (lanes 3 and 3a) either alone or in the presence of a 500-fold excess of unlabeled IL-8 (lane 1a), GROα(Y) (lanes 1b and 2a), or NAP-2(Y) (lanes 1c and 3a). As a control (lane C), cells were incubated with ^{125}I-labeled IL-8 in the absence of cross-linking agent. Molecular mass markers are as in Fig. 3.

for ^{125}I-labeled IL-8 in digitonin-solubilized preparations as compared with intact cells (K_d = 0.4 ± 0.3 nM vs. 0.18 ± 0.07 nM). A comparable increase in K_d was also observed for the high-affinity ^{125}I-labeled GROα(Y) receptors. The main effect of digitonin, however, was to decrease the number of high-affinity receptors for ^{125}I-labeled GROα(Y) and ^{125}I-labeled NAP-2(Y). As shown in Fig. 4*A*, only ≈10% of the total binding sites for GROα retained high-affinity (K_d = 1.0 nM), whereas high-affinity sites for NAP-2 could not be detected. After digitonin treatment both ligands were bound with a K_d value of 30 nM, which corresponds to the value calculated for the low-affinity receptors in intact cells (Fig. 2). Competition experiments using ^{125}I-labeled IL-8 and unlabeled IL-8, GROα(Y), or NAP-2(Y) indicated that the digitonin-solubilized receptors for IL-8 correspond to the low-affinity binding sites for GROα and NAP-2 (Fig. 4*B*). As implied by the sigmoidal competition curves, the experimental data could be best fitted to a single-site binding model. In this and similar experiments, <10% of the binding sites for GROα or NAP-2 were of high affinity (compare Figs. 4*B* and 1*C*).

In digitonin-solubilized receptor preparations a single prominent protein band of 40–46 kDa (p44) became cross-linked with ^{125}I-labeled IL-8, and this labeling was prevented by a 500-fold excess of unlabeled IL-8 (Fig. 5). Unlabeled GROα(Y) and NAP-2(Y) were much less effective in preventing the cross-linking with ^{125}I-labeled IL-8, reflecting the difference in binding affinity of this receptor for IL-8 and GROα or NAP-2. Prolonged autoradiography revealed a protein band of similar mobility (42–48 kDa) that was specifically cross-linked with ^{125}I-labeled GROα(Y) and ^{125}I-labeled NAP-2(Y). A 2- to 3-fold difference in the specific radioactivities of ^{125}I-labeled GROα(Y) and ^{125}I-labeled NAP-2(Y) could account for the observed difference in band intensity. In contrast to intact cells (Fig. 3), in these preparations, there was no evidence for the labeling of p70.

Effect of Guanine Nucleotides. Pretreatment of neutrophil membranes with 100 μM guanosine 5′-[γ-thio]triphosphate (GTP[γS]) reduced the affinity for IL-8 (K_d = 30 nM) in 60–65% (two experiments) of the binding sites, while the remaining receptors retained high affinity (K_d = 0.35 nM) (Fig. 6*A*). A similar effect was observed for the numbers of high-affinity receptors for GROα and NAP-2, which were reduced by 58–67% and 56–75% (two experiments), respectively (Fig. 6 *B* and *C*). After digitonin solubilization, however, no effect of GTP[γS] was observed, as shown for the receptors of IL-8, which fully retained high-affinity binding (Fig. 6*D*). Since only few or no high-affinity binding sites for GROα and NAP-2 were present in digitonin-solubilized receptor preparations, the effect of GTP[γS] on this binding could not be investigated. In control experiments, pretreatment of neutrophil membranes or digitonin-solubilized receptors with 100 μM ATP, another purine nucleotide, did not appreciably affect the binding of IL-8, GROα, and NAP-2.

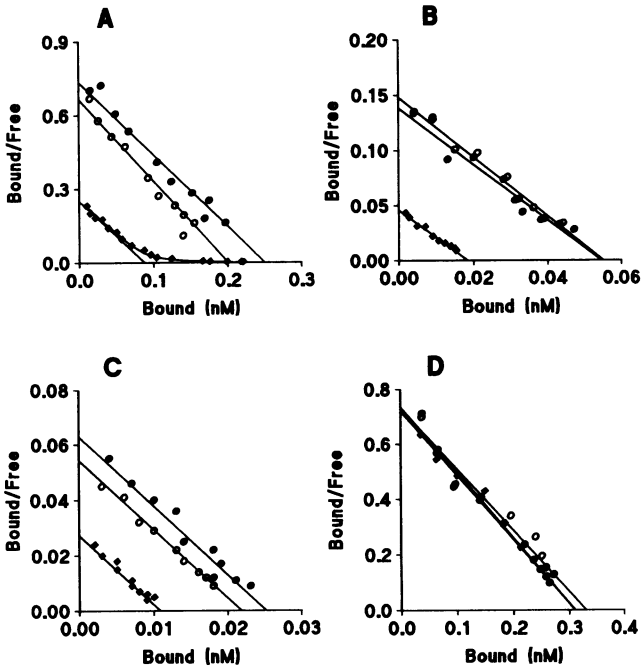

FIG. 6. Effect of GTP[γS] and ATP on receptor binding. Neutrophil membranes (*A–C*) or digitonin-solubilized receptor preparations (*D*) were pretreated with 100 μM GTP[γS] or ATP. Binding of ^{125}I-labeled IL-8 (*A* and *D*), ^{125}I-labeled GROα(Y) (*B*), and ^{125}I-labeled NAP-2(Y) (*C*) after pretreatment with 100 μM GTP[γS] (◆), 100 μM ATP (○), or buffer alone (●) is shown [1 nM bound corresponds to 12 fmol of ligand bound per μg of membrane protein (*A–C*) or 6 fmol of ligand bound per μg of soluble protein (*D*), and 1 unit of bound/free corresponds to 120 μl/10 μg of membrane and 120 μl/20 μg of soluble protein, respectively].

DISCUSSION

Structure–activity relationship studies with truncation analogs have demonstrated the critical involvement of the N terminus of IL-8 for receptor binding and neutrophil activation and have shown that several residues at the C terminus can be deleted without functional consequences (21). Accordingly, modification of the C termini with tyrosine residues of the IL-8 homologs, GROα and NAP-2, did not affect function and receptor binding. GROα(Y) and NAP-2(Y) bound to high- and low-affinity receptors on human neutrophils, and the binding of both ligands was prevented by unlabeled IL-8, indicating these receptors are shared by all three cytokines.

The relatedness of the receptors for GROα, NAP-2, and IL-8 as suggested by the binding analysis is also indicated by cross-linking experiments showing that radioiodinated GROα(Y), NAP-2(Y), and IL-8 specifically labeled two apparently identical protein bands (p44 and p70) in intact neutrophils. Additional evidence for the existence of common receptors for GROα, NAP-2, and IL-8 stems from intracellular calcium mobilization experiments where sequential stimulation of human neutrophils with the three cytokines led to cross-desensitization (11, 17).

The existence of two classes of IL-8 receptors was originally suggested by binding experiments showing that radiolabeled IL-8 could be displaced by high- and low-affinity competition with unlabeled GROα and NAP-2 (17). It is conceivable that the two proteins identified by cross-linking, p44 and p70, represent the high- and low-affinity receptors for

GROα and NAP-2, both of which bind IL-8 with high affinity. Interestingly, digitonin treatment of neutrophil membranes solubilized a receptor with low binding affinity for GROα and NAP-2, but high binding affinity for IL-8. Both, the high- and low-affinity binding constants were similar to the ones determined with intact cells. The results of the cross-linking experiments with digitonin-solubilized membrane preparations suggest that this receptor may correspond to p44.

Pretreatment with *Bordetella pertussis* toxin inhibits the motile and secretory responses of neutrophils to IL-8, indicating that G proteins of the G_i type are involved in signal transduction (22). In neutrophil membranes, the nonhydrolyzable GTP analog GTP[γS] was shown to lower the binding of fMet-Leu-Phe and C5a to the respective high-affinity receptors (23, 24). Our present results are in agreement with these findings. Under conditions where the effect of GTP[γS] was maximal (refs. 23 and 24 and C.S., unpublished observation), the affinity of about two-third of the receptors for IL-8, GROα, and NAP-2 was markedly reduced (by ≈75-fold) while the total number of binding sites was not affected. The partial effect of GTP[γS] could result from incomplete accessibility of the G proteins in our membrane vesicle preparations. Alternatively, part of the receptors for IL-8 and its two homologs may differ in their interaction with G proteins and/or regulation of ligand binding.

After solubilization with digitonin, 100 μM GTP[γS] had virtually no effect on the affinity and number of receptors for IL-8, which thus appear to differ from digitonin-solubilized C5a receptors. Siciliano *et al.* (23) reported that, even with low concentrations of GTP[γS] (1 μM as compared to 100 μM in our experiments), 90% of the C5a receptors were converted to low affinity, indicating that these receptors remained functionally associated with the respective G proteins after digitonin treatment. Digitonin-solubilized IL-8 receptors (corresponding to p44), by contrast, appear to be dissociated from G proteins and not to require G-protein coupling for high-affinity binding. This observation could be taken to suggest that neutrophil activation by IL-8 through p44 may depend on signal transduction mechanisms involving the coupling to different types of G_i-protein α subunits (25, 26). Affinity purification and characterization of these receptors in reconstitution experiments may help to address this question.

The present study demonstrates that human neutrophils bear two classes of IL-8 receptors. Both receptors bind IL-8 with high affinity but have different affinities for GROα and NAP-2. GROα and NAP-2 are significantly less potent than IL-8 as inducers of granule release and the respiratory burst in human neutrophils, whereas all three cytokines are nearly equipotent as stimuli of chemotaxis (7, 11, 12). The differences in receptor binding affinities, as described in this study, may explain the differences in the pattern of biological responses elicited by the three related cytokines.

We thank R. Stuber, Philip Owen, Peter Borowski, and Greg Radigan for technical assistance; Dr. V. von Tscharner for help with the computer modeling of the binding data; and Dr. B. Dewald for critical reading of the manuscript. Human donor blood buffy coats were provided by the Swiss Central Laboratory Blood Transfusion Service SRC. This work was supported by Grant 31-25700.88 from the Swiss National Science Foundation and the Protein Engineering Network of Centres of Excellence (PENCE); I.C.-L. is the recipient of a Scholarship from the Medical Research Council of Canada.

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