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Heterodimerization and cross-desensitization between the μ-opioid receptor and the chemokine CCR5 receptor

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Abstract

Cross-desensitization between μ-opioid receptor agonists and CC chemokines was shown to occur in immune cells and in the central nervous system. However, these cells do not permit examination of potential mechanisms at cellular levels due to low levels and mixed populations of receptors. In this study, we investigated possible interactions and biochemical mechanisms of cross-desensitization between the μ-opioid and chemokine CCR5 receptors coexpressed in Chinese hamster ovary (CHO) cells. Hemagglutinin (HA)-tagged μ-opioid receptor coimmunoprecipitated with FLAG (Asp–Tyr–Lys–Asp–Asp–Asp–Asp–Lys)-tagged chemokine receptor CCR5 in cells expressing the two receptors, but not in a mixture of cells transfected with one of the two receptors, indicating that the two receptors form heterodimers. Treatment with the μ-opioid receptor agonist DAMGO ([D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]-enkephalin), the chemokine RANTES (Regulated on Activation, Normal T cell-Expressed and -Secreted) (CCL5), or both, did not affect the level of coimmunoprecipitation. DAMGO and RANTES (CCL5) induced chemotaxis in CHO cells coexpressing both receptors, and preincubation with either DAMGO or RANTES (CCL5) profoundly inhibited chemotaxis caused by the other. DAMGO pretreatment enhanced phosphorylation of the chemokine CCR5 receptor and reduced RANTES (CCL5)-promoted [^{35}S]GTPγS binding. Conversely, RANTES (CCL5) preincubation slightly increased phosphorylation of the μ-opioid receptor and significantly reduced DAMGO-induced [^{35}S]GTPγS binding. These results indicate that activation of either receptor affected G protein coupling of the other, likely due to enhanced phosphorylation of the receptor. Heterodimerization between the two receptors may contribute to the observed cross-desensitization.

Keywords: μ-Opioid receptor; CCR5 chemokine receptor; Heterodimer; Cross-desensitization

1. Introduction

Opiates and opioids interact with specific receptors to produce unique physiological and pharmacological effects, most notably modulation of pain perception and regulation of mood. The presence of at least three types of opioid receptors—μ, δ, and κ—was established in the 1970s and 1980s by pharmacological, binding, and anatomical distribution analyses. μ, δ, and κ opioid receptors have been cloned (for reviews, see Kieffer, 1995; Knapp et al., 1995). Activation of opioid receptors, coupled via pertussis toxin (PTX)-sensitive G proteins, induces a number of cellular effects, including inhibition of adenylyl cyclase, increase in K^+ conductance and decrease in Ca^{2+} conductance, and stimulation of the p42/p44 mitogen-activated protein (MAP) kinase pathways (for a review, see Law et al., 2000).

Exposure to opiates and opioids has been reported to inhibit cellular immune responses and induce chemotactic responses in immune cells (for reviews, see Peterson et al., 1998; McCarthy et al., 2001). These effects on the immune system may contribute to impaired immune function (Des Jarlais et al., 1988) and a higher rate of infection by the human immunodeficiency virus (HIV) in chronic heroin users (Holmberg, 1996). These effects are, at least in part, mediated by opioid receptors as the mRNAs of μ, δ, and κ opioid receptors have been shown to be present in immune cells (for a review, see McCarthy et al., 2001).

Chemokines and chemokine receptors play crucial roles in the functions of the immune system, including leukocyte

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development, and function and clearance of infectious organisms. Chemokine receptors CCR5 and CXCR4 act as major coreceptors, along with CD4, for the entry of HIV into cells (Cairns and D'Souza, 1998). In addition, recent studies have shown that chemokine and chemokine receptors are present in the central nervous system and play important roles in brain development, regulation of neurotransmission, and pathophysiological states in which inflammation persists (Bajetto et al., 2002). To date, 19 chemokine receptors have been identified, and many chemokines bind to multiple receptors and most chemokine receptors recognize several chemokines (for a review, see Proudfoot, 2002). Activation of chemokine receptors, coupled via G proteins ($G_{i/o}$, G_q, and G_{16}) (Arai and Charo, 1996), results in cellular effects such as inhibition of adenylyl cyclase (Zhao et al., 1998); stimulation of phospholipases A, C, and D; activation of p42/p44 MAP kinases; stimulation of phosphatidylinositol-3-kinase; and enhancement of nonreceptor tyrosine kinase activities (for reviews, see Bokoch, 1995; Maghazachi, 1999).

Opioid treatment of immune cells desensitized the chemotactic response induced by several chemokines; in turn, pretreatment with some chemokines reduced the chemotaxis induced by some opioids (Grimm et al., 1998a,b; Choi et al., 1999; Rogers et al., 2000; Miyagi et al., 2000). Heterologous desensitization of CCR5 may reduce susceptibility to HIV infection (Cairns and D'Souza, 1998; Shen et al., 2000; Szabo et al., in press). In addition, administration of some chemokines into the periaqueductal gray of the rat brain reduced the antinociceptive effects of μ-opioid receptor agonists (Szabo et al., 2002). Cross-talk between the opioid and chemokine systems may serve as a modulatory mechanism to fine-tune cellular function (for a review, see McCarthy et al., 2001).

Like many other G protein-coupled receptors (GPCRs), both the μ-opioid and chemokine CCR5 receptors are desensitized following prolonged agonist exposure (Zhang et al., 1996; Aramori et al., 1997). Two major types of desensitization have been characterized: homologous and heterologous. In homologous desensitization, only the activated receptor is desensitized, while in heterologous desensitization, activation of a receptor causes reduced responsiveness of another receptor. Homologous desensitization of GPCRs shares similar mechanisms; however, the mechanisms underlying heterologous desensitization of GPCRs are less uniform. Multiple processes may be involved in heterologous desensitization, including changes at the levels of receptors, G proteins, and second messenger pathways (Ali et al., 1999; Willars et al., 1999).

GPCRs have been shown to form dimers with the same receptor or a different receptor (for reviews, see Milligan, 2001; Devi, 2001; Angers et al., 2002). μ-Opioid receptors undergo heterodimerization with several GPCRs including the δ-opioid and β_2-adrenergic receptors. The ligand binding, signaling properties, and cellular function of a number of GPCRs have been reported to be modified as a result of receptor dimerization (for reviews, see Milligan, 2001; Devi, 2001; Angers et al., 2002).

In the present study, we investigated whether the μ-opioid and the chemokine CCR5 receptors formed heterodimers, and examined the early cellular signaling events following agonist binding, which may contribute to receptor cross-desensitization. We were not able to conduct such studies in immune cells and the central nervous system where cross-desensitization between opioids and chemokines has been reported, since these cells contain low levels of a heterogeneous population of opioid and chemokine receptors. The low levels of receptors do not permit biochemical characterization and the presence of several different chemokine and/or opioid receptors does not lend themselves to the unequivocal characterization of actions at a specific receptor type. We thus used a cell model, Chinese hamster ovary (CHO) cells stably coexpressing the CCR5 and μ-opioid receptors, for the study.

2. Materials and methods

2.1. Materials

[^{35}S]GTPγS (~ 1250 Ci/mmol), [^{3}H]diprenorphine (~ 58 Ci/mmol), and [^{32}P]orthophosphate (~ 8500 Ci/mmol) were purchased from Perkin Elmer Life Science (Boston, MA) and [^{125}I]MIP-1â was from Amersham Pharmacia Biotech (Piscataway, NJ). Naloxone was a gift from DuPont/Merck (Willmington, DE). Rabbit anti-FLAG polyclonal antibody (F-7425), Dulbecco's modified Eagle's medium, GDP, and GTPγS were obtained from Sigma (Louis, MO). The following reagents were purchased from the indicated companies: phospho-p44/42 MAPK antibody and HRP-conjugated antirabbit IgG (γ-immunoglobulin) from New England Biolaboratories (Beverly, MA); DAMGO from Research Biochemicals International (Natick, MA); human RANTES (CCL5) from PeproTech (Rocky Hill, NJ); 48-well microchemotaxis chamber from NeuroProbe (Gaithersburg, MD); Pansorbin from Calbiochem (San Diego, CA); geneticin from Mediatech (Herndon, VA); fetal calf serum from Hyclone (Logan, UT); Lipofectamine, hygromycin B, penicillin, streptomycin, and Hank's balanced salt solution from Invitrogen (Carlsbad, CA); Complete Mini Protease Inhibitor Cocktail Tablets™, M1, and M2 anti-FLAG monoclonal antibodies from Roche Diagnostics (Mannheim, Germany); Super-Signal chemiluminescent reagent from Pierce (Rockford, IL); PE-conjugated anti-CCR5 (2D7/CCR5) antibody from BD Pharmingen (San Diego, CA); mouse anti-hemagglutinin (HA) monoclonal antibody (HA.11), horseradish peroxidase-conjugated goat antimouse, and antirabbit IgG from Jackson ImmunoResearch Laboratory (West Grove, PA).

The human chemokine receptor CCR5 cDNA clone was obtained from the AIDS Research and Reference Reagent

Program, Division of AIDS, NIAID, NIH. The clone was originally donated by Dr. Nathaniel Landau of the Aron Diamond AIDS Research Center, The Rockefeller University. The CCR5 cDNA was epitope-tagged with FLAG at the N-terminus. The rat μ-opioid receptor cDNA clone (Chen et al., 1993) was a gift from Dr. Lei Yu of the University of Cincinnati and was tagged with HA at the N-terminus (Xu et al., 1999).

2.2. Coimmunoprecipitation of the μ-opioid and chemokine CCR5 receptors

CHO cells stably expressing HA-tagged rat μ-opioid receptor (HA-μ) (μ72) were transiently transfected with the FLAG-CCR5 (FLAG-tagged chemokine CCR5 receptor) cDNA with Lipofectamine. In parallel, CHO cells were similarly transfected with the FLAG-CCR5 cDNA. Forty-eight hours after transfection, cells transfected with both μ-opioid and chemokine CCR5 receptors (CHO-HA-μ/FLAG-CCR5) and a mixture of CHO-HA-μ and CHO-FLAG-CCR5 cells were harvested and solubilized in NIT solution (1.2% NP-40, 0.1 M iodoacetamide, 0.15 M NaCl, 20 mM Tris HCl, pH 7.4, and Complete Protease Inhibitor Cocktail™) for 1 h at 4 °C. The mixtures were centrifuged and filtered through 0.22-μM membranes, and the solubilized materials were incubated with rabbit polyclonal antibodies against FLAG (F7425) at 5 μg/ml for 1 h at 4 °C followed by Pansorbin (final 1/200) at 4 °C for 1 h. The mixture was centrifuged and the pellets were washed three times by centrifugation and resuspension. Immunoprecipitated materials were dissolved in Lammeli sample buffer containing 0.1 M DTT (dithiothreitol), subjected to 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Chen et al., 1995), and transferred onto nitrocellulose membranes. Nitrocellulose membranes were treated with blocking solution and incubated with a monoclonal antibody against HA at 1:2000 dilution, and then goat antimouse IgG was conjugated with horseradish peroxidase at 1:4000 and reacted with chemiluminescence Western blotting detection reagents. The nitrocellulose membranes were then stripped and immunoblotting was carried out to assess the amount of FLAG-CCR5 immunoprecipitated with M1 mouse monoclonal antibodies against FLAG and goat antimouse IgG conjugated with horseradish peroxidase. In addition, Western blot was performed on the solubilized materials (without immunoprecipitation) with a monoclonal antibody against HA to assess the amounts of the HA rat μ-opioid receptor in the coexpressing cells and in the cell mixtures.

In a separate series of experiments, HA rat μ-opioid receptor was immunoprecipitated with rabbit antiserum against a C-terminal domain peptide (383–398) of the rat μ-opioid receptor (anti-RMOR) (1:500) (Chen et al., 1996) and immunoblotting was performed with M2 mouse monoclonal antibodies against FLAG (1:2000) according to the procedure described above.

2.3. Stable coexpression of the μ-opioid and FLAG-CCR5 receptors in CHO cells

CHO cell clones stably expressing the rat μ-opioid receptor in the vector pcDNA3/neomycin (Li et al., 1999) were transfected with the FLAG-CCR5 in the vector pcDNA3/hygromycin B using Lipofectamine. Forty-eight hours after transfection, cells were grown under the selection pressure of geneticin (1 mg/ml) and hygromycin (0.5 mg/ml) in DMEM/F12 (Dulbecco's modified Eagle's medium/F12 HAM) supplemented with 10% fetal calf serum in a humidified atmosphere with 5% CO_2 at 37 °C. Two weeks later, cells were transferred into 96-well plates at an average of 1 cell/well and, upon reaching confluence, clonal cells were transferred into 24-well plates in quadruplicate (three for experiments and one in a separate plate for propagation). Agonist-induced p42/p44 MAP kinase phosphorylation was used to screen for stable expression of both receptors. Briefly, when cells were about 80% confluent, the medium was aspirated and cells were washed, cultured in DMEM/F12 supplemented with 0.5% fetal calf serum, and incubated at 37 °C with 5% CO_2 for 2 h to reduce basal p42/p44 MAP kinase phosphorylation. DAMGO (10 μM), RANTES (CCL5) (10 nM), or medium was added to one well each and incubated for 10 min. The ligands were removed by aspiration and 2× Laemmli sample buffer was added to the three wells [DAMGO, RANTES (CCL5), and blank], transferred to 1.5-ml Eppendorf tubes, and boiled immediately. Samples were analyzed by Western blot using phospho-p44/42 MAPK antibody according to the vendor's instructions (New England Biolaboratories). Clonal cells showing enhanced p44/42 MAP kinase phosphorylation by DAMGO and RANTES (CCL5) indicate coexpression of MOR and FLAG-CCR5, which was further confirmed by receptor binding with [^{3}H]diprenorphine and [^{125}I]macrophage inflammatory protein-1β (MIP-1β). Selected clonal cells were maintained in DMEM/F12 with 0.5 mg/ml geneticin and 0.2 mg/ml hygromycin. Cells were harvested for experiments by use of Versene solution (0.54 mM ethylenediaminetetraacetic acid, 0.14 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.46 mM KH_2PO_4, 1 mM glucose, pH 7.0).

2.4. Opioid receptor binding and internalization

Cell membranes were prepared as previously reported (Chen et al., 1996). Binding was carried out in 50 mM Tris–HCl buffer containing 1 mM EGTA (pH 7.4) at room temperature for 1 h in duplicate in a final volume of 1 ml with 10–20 μg of membrane protein. Saturation binding of [^{33}H]diprenorphine to the μ-opioid receptor was performed with at least six concentrations of [^{33}H]diprenorphine (ranging from 25 pM to 2 nM), and K_d and B_{max} values were determined. Competitive inhibition of [^{3}H]diprenorphine binding to the μ receptor was performed with 0.4 nM

[^{3}H]diprenorphine in the absence or presence of different concentrations of DAMGO, and K_i values of DAMGO were determined. Naloxone (1 μM) was used to define nonspecific binding. Binding data were analyzed with the EBDA program (McPherson, 1983).

For internalization studies, receptor binding was performed on intact cells with ~1 nM [^{3}H]diprenorphine in phosphate-buffered saline (PBS; pH 7.2). Nonspecific binding was defined with 1 μM naloxone for the total receptors and 5 μM CTAP (Cys^2, Tyr^3, Arg^5, and Pen^{7}Amide) for the cell surface receptors. The difference between the total receptors and the cell surface receptors represents intracellular receptors.

2.5. CCR5 binding

CCR5 binding assay was performed on intact cells according to a modification of the procedure described by Samson et al. (1996). Cells (7.5×10^4 cells/tube) were incubated with [^{125}I]MIP-1β (0.1 nM) and various concentrations of unlabeled MIP-1β (from 2 to 64 nM) at room temperature for 45 min, centrifuged at 4000 × *g* at 4 °C for 5 min, and washed two times by resuspension and centrifugation. Radioactivity associated with cells was measured in a gamma counter and binding data were analyzed with the EBDA program (McPherson, 1983).

2.6. Chemotaxis

The analysis of chemotaxis was performed by standard procedures (Grimm et al., 1998a) in a 48-well microchemotaxis chamber. Briefly, cells were incubated in RPMI-1640 medium containing 1% bovine serum albumin (BSA) and 25 mM HEPES in the upper chamber, and the chemoattractant was loaded in the bottom chamber in the same medium, separated by a polyvinylpyrrolidone-free 5-μm pore size membrane. Migration in response to DAMGO or RANTES (CCL5) was allowed for 90–180 min at 37 °C in 5% CO_2. The membranes were then removed from the chamber, the upper side was washed and scraped, and the membranes were fixed and stained. The results are expressed as the chemotaxis index (mean number of cells per high-power field for chemoattractant dilution/mean number of cells for the medium control).

2.7. [^{35}S]GTPγS binding

Cells were treated with or without the chemokine agonist RANTES (CCL5) or the μ-opioid receptor agonist DAMGO for an indicated period at 37 °C. Cells were then collected and membranes were prepared in the presence of 10 mM NaF and 10 mM Na pyrophosphate to inhibit phosphatases (Zhu et al., 1998). [^{35}S]GTPγS binding was performed as described previously (Li et al., 2001). Briefly, membranes (containing 10–20 μg of proteins) were incubated with 15 μM GDP and ~0.2 nM [^{35}S]GTPγS in the presence or absence of a drug in a reaction buffer (50 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, and 0.1% BSA) in a final volume of 0.5 ml. Nonspecific binding was determined in the presence of 10 μM GTPγS. After 60 min of incubation at 30 °C, bound and free [^{35}S]GTPγS were separated by filtration with GF/B filters under reduced pressure and the filter was washed. Radioactivity in filters was determined by liquid scintillation counting.

2.8. Phosphorylation of opioid and chemokine receptors

Phosphorylation of opioid and chemokine receptors was conducted according to a procedure described previously (Carman et al., 2000). CHO-MOR/FLAG-CCR5 cells were grown to confluence in six-well plates and two wells of cells were combined for each assay. Cells were washed twice with phosphate-free DMEM and incubated at 37 °C for 2 h with 1 ml/well phosphate-free DMEM containing 500 μCi/ml [^{32}P]orthophosphate. RANTES (CCL5) or DAMGO was then added and incubated for 10 min at 37 °C and washed three times with ice-cold PBS. All subsequent steps were carried out at 4 °C. Cells were solubilized for 2 h with 0.4 ml/well of solubilization buffer (2% digitonin, 0.5% sodium deoxycholate, 5 mM EDTA, 10 mM sodium pyrophosphate, 10 mM NaF, 20 nM calyclulin A, and 1 tablet/10 ml Complete Protease Inhibitor Cocktail™). Solubilized mixtures were centrifuged and the supernatants were precleared by incubation with Pansorbin and normal rabbit serum for 1 h. For immunoprecipitation of the opioid or chemokine receptors, the supernatant was incubated overnight with rabbit antiserum against a C-terminal domain peptide (383–398) of the rat μ-opioid receptor (2 μl for 0.8 ml) (Chen et al., 1996) and the rabbit anti-FLAG antibodies (4 μg for 0.8 ml), respectively, followed by Pansorbin (final 1/200) at 4 °C for 1 h. The suspension was centrifuged at 9000 × *g* and the pellet was washed three times with solubilization solution by centrifugation and resuspension. The pellet was dissociated in 2 × Lammeli sample buffer and subjected to 7% SDS-PAGE and autoradiography.

2.9. Internalization of FLAG-CCR5

CHO-MOR/FLAG-CCR5 cells were harvested, washed, and resuspended in a medium containing RPMI-1640, 25 mM HEPES, glutamine, and 1% BSA. Cells were treated with 1 μM DAMGO or 12.5 nM RANTES (CCL5) and incubated for 30 min at 37 °C, washed with cold Hank's balanced salt solution with 2% endotoxin-free fetal calf serum, and resuspended in the same solution. Subsequently, cells were incubated with normal goat serum at 4 °C for 30 min to block nonspecific binding. Cells were treated with PE-conjugated anti-CCR5 (2D7/CCR5) antibody, incubated at 4 °C for 45 min, washed, and analyzed in a Coulter Epics XL flow cytometer (Coulter, Hialeah, FL).

3. Results

3.1. Heterodimerization of the μ-opioid receptor and the chemokine receptor CCR5

CHO-HA-μ cells expressed ~0.5 pmol/mg protein of HA-tagged μ-opioid receptor as determined by [^{3}H]diprenorphine binding. FLAG-CCR5 cDNA was transfected into CHO or CHO-HA-μ cells to approximately 1 pmol/10^6 cells. CHO-HA-μ/FLAG-CCR5 cells, or a mixture of CHO-HA-μ and CHO cells transfected with the FLAG-CCR5 receptor was solubilized and incubated with anti-FLAG antibody to immunoprecipitate FLAG-CCR5, and immunoprecipitated materials were resolved by SDS-PAGE followed by immunoblotting with anti-HA antibody to detect the HA-μ receptor. In cells coexpressing the HA-μ and FLAG-CCR5 receptors, the μ-opioid receptor coimmunoprecipitated with the chemokine receptor CCR5 (Fig. 1A), indicating formation of heterodimers or oligomers. In contrast, in a mixture of cells individually expressing HA-μ or FLAG-CCR5 receptors, the μ receptor was not coimmunoprecipitated with the CCR5 (Fig. 1A), even though the level of the μ-opioid receptor and the amount of immunoprecipitated CCR5 were similar (Fig. 1B and C). These results indicate that that the heterodimers were not formed during solubilization/immunoprecipitation procedures.

To confirm the finding, we carried out experiments using rabbit antiserum against a peptide of the C-terminal domain (383–398) of the rat μ-opioid receptor (Chen et al., 1996) to immunoprecipitate the μ-opioid receptor, followed by immunoblotting with a mouse monoclonal antibody against the FLAG epitope. In CHO cells expressing both HA-μ and FLAG-CCR5, there was a FLAG-immunoreactive protein band (Fig. 1D), indicating that FLAG-CCR5 immunoprecipitates with the μ-opioid receptor. In contrast, in mixtures of CHO cells expressing HA-μ or FLAG-CCR5 individually, there was no FLAG-immunoreactive band (Fig. 1D).

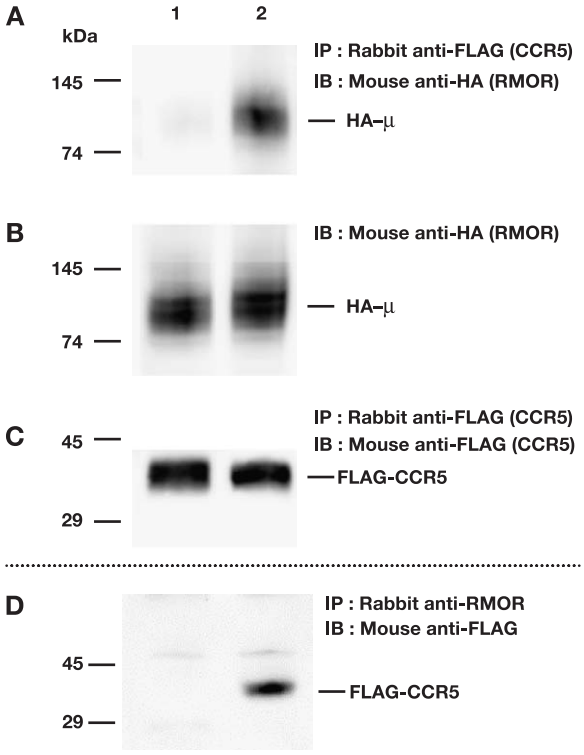

Fig. 1. Coimmunoprecipitation of the chemokine CCR5 receptor and the μ-opioid receptor in CHO cells cotransfected with both receptors. CHO cells or CHO cells stably expressing HA-μ were transiently transfected with the cDNA construct of the FLAG-CCR5 receptor. Forty-eight hours later, CHO-HA-μ/FLAG-CCR5 cells (lane 2) and a mixture of CHO-HA-μ and CHO-FLAG-CCR5 cells (Lane 1) were solubilized. (A) Solubilized materials were immunoprecipitated with rabbit anti-FLAG antibody (F7425) and Pansorbin. Immunoprecipitated materials were dissolved in Lammeli sample buffer containing 0.1 M DTT and resolved with SDS-PAGE; immunoblotting was performed with a monoclonal antibody against HA; and the goat antimouse IgG was conjugated with horseradish peroxidase and reacted with enhanced chemiluminescence detection reagents. (B) Solubilized materials were resolved with SDS-PAGE and immunoblotting was carried out with a monoclonal antibody against HA to detect the HA-μ-opioid receptor. Note that the levels of the μ receptor were similar in both lanes. (C) Membranes shown in (A) were stripped and reblotted with a monoclonal antibody against FLAG to detect immunoprecipitated FLAG-CCR5 receptor. Note that the levels of immunoprecipitated CCR5 receptor were similar in both lanes. (D) Solubilized materials were immunoprecipitated with rabbit anti-RMOR(383–398) and resolved on SDS-PAGE, and immunoblotting was carried out with M2 mouse monoclonal antibody against FLAG to detect FLAG-CCR5. The chemokine CCR5 receptor was detected in CHO-HA-μ/FLAG-CCR5 cells (lane 2), but not in the mixture of CHO cells expressing HA-μ or FLAG-CCR5 individually (lane 1). Each figure represents one of the three or four experiments performed with similar results.

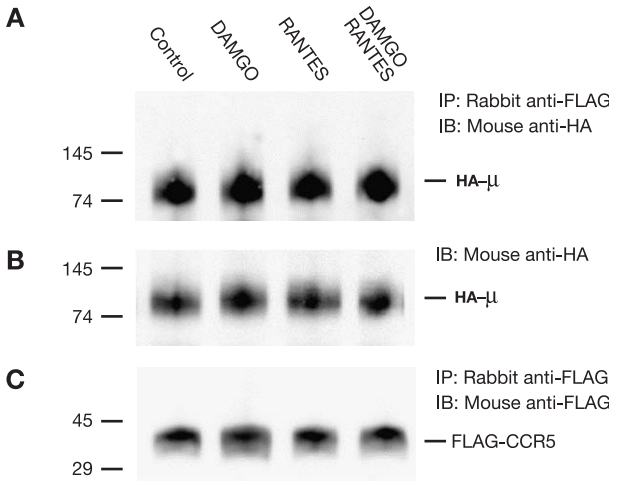

Fig. 2. Effects of agonist treatment on coimmunoprecipitation of the chemokine CCR5 receptor and the μ-opioid receptor. CHO cells stably transfected with HA-μ were transiently transfected with the cDNA construct of FLAG-CCR5. Forty-eight hours later, cells were left untreated or treated at 37 °C with DAMGO (1 μM), RANTES (CCL5) (12.5 nM or 100 ng/ml), or DAMGO (1 μM) and RANTES (CCL5) (12.5 nM) for 30 min, washed, detached with ice-cold Versene, and solubilized. (A–C) Solubilized materials were subjected to immunoprecipitation and immunoblotting as described in Fig. 1A–C. The figure represents one of the three experiments performed with similar results.

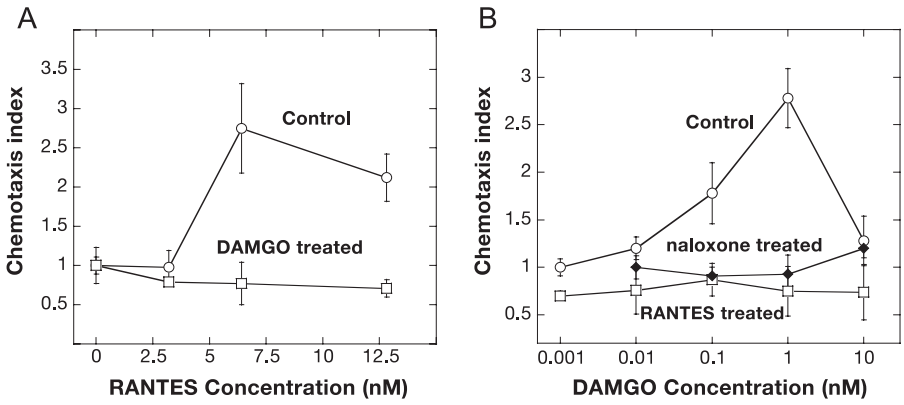

Fig. 3. Bidirectional cross-desensitization of the μ-opioid receptor and the chemokine receptor CCR5 in agonist-induced chemotaxis in CHO-MOR/FLAG-CCR5 cells. Cells were pretreated with either (A) DAMGO (10 nM) or (B) RANTES (CCL5) (100 ng/ml, 12.5 nM) or naloxone (1 μM) for 30 min. Migration of cells in response to (A) RANTES (CCL5) or (B) DAMGO was assayed as described in Materials and Methods. Results are expressed as chemotaxis index [mean number of cells per high-power field for DAMGO or RANTES (CCL5)/mean number of cells per high-power field for medium]. Each value represents the mean ± S.E.M. of three or four independent experiments performed in duplicate.

Treatment of CHO-HA-μ/FLAG-CCR5 cells with DAMGO, RANTES (CCL5), or both did not change the amount of the μ-opioid receptor coimmunoprecipitated with the chemokine CCR5 receptor (Fig. 2A). The level of the μ-opioid receptor and the amount of immunoprecipitated chemokine CCR5 receptor were similar in the control and treatment groups (Fig. 2B and C).

3.2. Establishment of CHO-MOR/FLAG-CCR5 cell lines

In our experience, stably transfected cell lines, but not transiently transfected cells, yielded consistent results for chemotactic responses, [^{35}S]GTPγS binding, and receptor phosphorylation. We thus established CHO-MOR/FLAG-CCR5 cell lines to assess the functional cross-desensitization between the chemokine receptor CCR5 and the μ-opioid receptor. Saturation binding with the radioligand [^{3}H]diprenorphine demonstrated a K_d value of 0.2 nM and a B_{max} value of 2.2 pmol/mg membrane protein for the rat μ-opioid receptor. In addition, saturation binding of the agonist [^{125}I]MIP-1β to the CCR5 in intact cells exhibited a K_d value of 13.1 nM and a B_{max} of 1.43 pmol/10^6 cells. No specific radioligand binding was detected in untransfected CHO cells and no ligand cross-reactivity could be detected in single-receptor transfected CHO cells (data not shown).

Table 1
Effect of DAMGO pretreatment on K_d and B_{max} values of [^{125}I]MIP-1β binding to CCR5 in CHO-MOR/FLAG-CCR5 cells

	K_d (nM)	B_{max} (pmol/10^6 cell)
Control	13.1 ± 1.47	1.43 ± 0.18
DAMGO-treated	11.9 ± 0.42	1.38 ± 0.11

Intact cells were preincubated with or without 1 μM DAMGO for 30 min at 37 °C. Binding was performed with 0.1 nM [^{125}I]MIP-1β and 2–64 nM MIP-1β (seven concentrations) at room temperature for 45 min, and K_d and B_{max} values were determined. Data are expressed as mean ± S.E.M. of three independent experiments performed in duplicate.

3.3. Chemotaxis

CHO-MOR/FLAG-CCR5 cells exhibited robust chemotactic responses to both RANTES (CCL5) and DAMGO (Fig. 3). CHO-MOR/FLAG-CCR5 cells failed to manifest a chemotactic response to DAMGO following pretreatment with the opioid receptor antagonist, naloxone (Fig. 3B), indicating that the chemotactic response to DAMGO is mediated by the μ-opioid receptor. In contrast, naloxone failed to alter the chemotactic response induced by RANTES (CCL5). CHO-MOR/FLAG-CCR5 cells pretreated with DAMGO failed to exhibit a chemotactic response to RANTES (CCL5) (Fig. 3A). Likewise, CHO-MOR/FLAG-CCR5 cells preincubated with RANTES (CCL5) failed to manifest a chemotactic response to DAMGO (Fig. 3B).

3.4. Receptor affinity and number

Next we examined whether changes in receptor affinity and number occurred during cross-desensitization. Pretreatment of CHO-MOR/FLAG-CCR5 cells with DAMGO did not affect the K_d and B_{max} values of [^{125}I]MIP-Iβ binding to CCR5 in intact cells (Table 1). In addition, RANTES (CCL5) pretreatment did not change significantly either

Table 2
Effect of RANTES (CCL5) pretreatment on K_i and B_{max} values of DAMGO in inhibiting [^{3}H]diprenorphine binding to the μ-opioid receptor in membranes

	K_i (nM)	B_{max} (pmol/mg protein)
Control	4.94 ± 1.38	2.20 ± 0.10
RANTES (CCL5)-treated	5.19 ± 1.26	2.27 ± 0.15

CHO-MOR/FLAG-CCR5 cells were pretreated with 6.25 nM (50 ng/ml) RANTES (CCL5) for 30 min at 37 °C and membranes were prepared. Competitive inhibition by DAMGO of [^{3}H]diprenorphine binding to the μ-opioid receptor was performed on membranes, and K_i and B_{max} values were calculated. Results are expressed as mean ± S.E.M. of four independent experiments performed in duplicate.

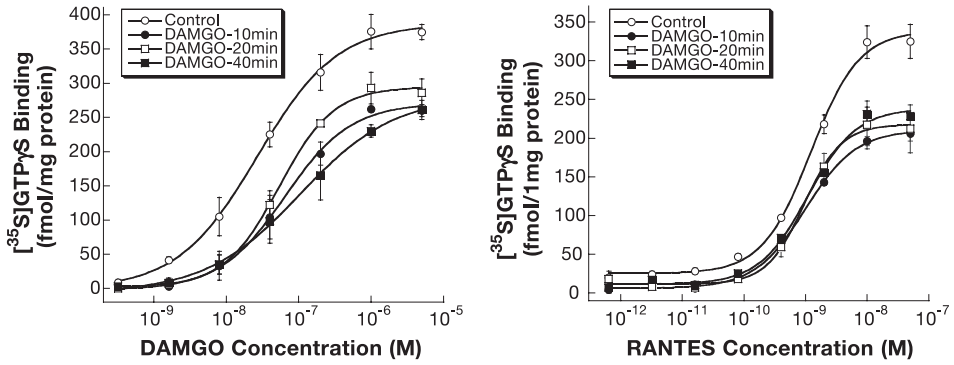

Fig. 4. Effects of DAMGO pretreatment on RANTES (CCL5)- and DAMGO-stimulated [^{35}S]GTPγS binding. CHO-MOR/FLAG-CCR5 cells were incubated without (control) or with 1 μM DAMGO for 10, 20, or 40 min at 37 °C. Cells were washed, membranes were prepared, and [^{35}S]GTPγS binding was performed in the presence of different concentrations of DAMGO or RANTES (CCL5) as described in Materials and Methods. Each value represents the mean ± S.E.M. of three or four independent experiments performed in duplicate. Basal [^{35}S]GTPγS binding (~ 150 fmol/mg protein) did not differ among the control and the treatment groups and was subtracted from the binding data. The EC_{50} and maximal levels of [^{35}S]GTPγS binding for RANTES (CCL5) and DAMGO are presented in Table 3.

the K_d or B_{max} of [^{3}H]diprenorphine binding to the μ-opioid receptor (data not shown), or the K_i or B_{max} values of DAMGO binding to the μ-opioid receptor in membranes (Table 2).

3.5. Receptor–G protein coupling

[^{35}S]GTPγS binding assay has been widely utilized to assess the agonist-dependent activation of PTX-sensitive G proteins mediated by a number of GPCRs including the μ-opioid and chemokine CCR5 receptors (Traynor and Nahorski, 1995; Zhao et al., 1998). We examined whether receptor–G protein coupling was affected during cross-desensitization. Both RANTES (CCL5) and DAMGO induced a dose-dependent increase in [^{35}S]GTPγS binding (Figs. 4 and 5), whereas no significant DAMGO- or RANTES (CCL5)-dependent effect upon [^{35}S]GTPγS binding was detected in untransfected CHO cells.

Pretreatment of CHO-MOR/FLAG-CCR5 cells with DAMGO reduced the maximal responses of RANTES (CCL5)- and DAMGO-promoted [^{35}S]GTPγS binding and increased the EC_{50} value of DAMGO, without affecting the EC_{50} value of RANTES (CCL5) (Fig. 4, Table 3). In contrast, pretreatment of cells expressing only FLAG-CCR5 with DAMGO did not affect the EC_{50} or B_{max} values of RANTES (CCL5) in enhancing [^{35}S]GTPγS binding (data not shown).

Preincubation with RANTES (CCL5) attenuated the maximal responses of RANTES (CCL5)- and DAMGO-promoted [^{35}S]GTPγS binding and increased the EC_{50} value of RANTES (CCL5), without affecting the EC_{50} value of DAMGO (Fig. 5, Table 4). Increasing pretreatment intervals (from 10 to 20 or 40 min) did not enhance the extent of desensitization (Figs. 4 and 5, Tables 3 and 4). However, preincubation of cells expressing the μ-opioid receptor alone with RANTES (CCL5) did not affect the EC_{50} or B_{max}

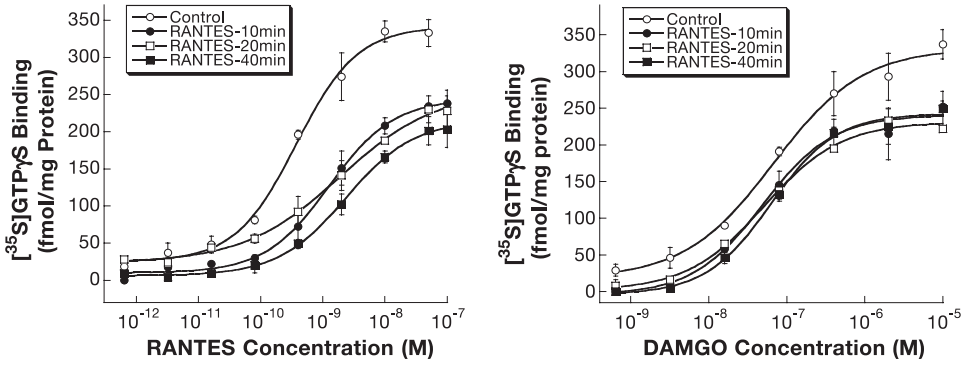

Fig. 5. Effects of RANTES (CCL5) pretreatment on DAMGO- and RANTES (CCL5)-promoted [^{35}S]GTPγS binding. CHO-MOR/FLAG-CCR5 cells were treated without (control) or with 6.25 nM (50 ng/ml) RANTES (CCL5) at 37 °C for 10, 20, or 40 min. Cells were washed, membranes were prepared, and [^{35}S]GTPγS binding was performed in the presence of different concentrations of DAMGO or RANTES (CCL5). Each value represents mean ± S.E.M. of three or four independent experiments in duplicate. Basal [^{35}S]GTPγS binding (~ 150 fmol/mg protein) did not differ among the control and the treatment groups, and was subtracted from the binding data. EC_{50} and maximal binding of [^{35}S]GTPγS binding induced by RANTES (CCL5) and DAMGO are shown in Table 4.

Table 3
EC_{50} and E_{max} values of RANTES (CCL5) and DAMGO in promoting [^{35}S]GTPγS binding following DAMGO pretreatment

DAMGO-pretreated	RANTES (CCL5)			DAMGO		
	EC_{50} (nM)	E_{max} (fmol/mg protein)	n	EC_{50} (nM)	E_{max} (fmol/mg protein)	n
Control	1.20 ± 0.22	323.0 ± 52.6	3	66.5 ± 12.1	329.0 ± 14.5	4
10 min	0.97 ± 0.05	198.7 ± 14.7	3	173.2 ± 23.1	230.3 ± 10.1	3
20 min	0.89 ± 0.12	189.3 ± 17.1	3	211.1 ± 38.2	253.8 ± 6.6	4
40 min	1.28 ± 0.11	190.7 ± 16.1	3	247.9 ± 18.9	240.3 ± 16.1	4

See Fig. 4 legend.

values of DAMGO in enhancing [^{35}S]GTPγS binding (data not shown).

These results indicate that pretreatment with either DAMGO or RANTES (CCL5) reduces the ability of the μ-opioid and chemokine CCR5 receptors to activate G proteins.

3.6. Receptor phosphorylation

Attenuation of receptor–G protein coupling may be due, in part, to receptor phosphorylation, which has been implicated in heterologous desensitization (Ali et al., 1999). We thus examined whether RANTES (CCL5) or DAMGO treatment could induce phosphorylation of the μ-opioid and/or chemokine CCR5 receptor(s). Preincubation with DAMGO, in addition to enhancing the phosphorylation of the μ-opioid receptor, significantly elevated phosphorylation of the CCR5 (Fig. 6). Likewise, RANTES (CCL5) pretreatment increased phosphorylation of both the CCR5 and the μ-opioid receptor (Fig. 6). However, the extent of phosphorylation of the μ-opioid and chemokine CCR5 receptors induced by RANTES (CCL5) and DAMGO, respectively, was less than those induced by the cognate agonists (Fig. 6). Molecular weight ranges of the μ-opioid and chemokine CCR5 receptors were similar to those previously reported (Chen et al., 1995; Zhang et al., 1996; El Kouhen et al., 1999; Oppermann et al., 1999; Carman et al., 2000).

3.7. Receptor internalization

Pretreatment with DAMGO for 30 min caused internalization of 25 ± 5% (mean ± S.E.M., $n = 4$) of the μ receptor in MOR/FLAG-CCR5 cells and a similar extent in MOR cells as determined by receptor binding, but it did not affect the staining of FLAG-CCR5 on cell surface of cells expressing MOR/FLAG-CCR5 or FLAG-CCR5 alone by immunofluorescence flow cytometry. Etorphine, a nonselective opioid receptor agonist, yielded similar results (data now shown). In addition, following RANTES (CCL5) pretreatment, the numbers of total and cell surface μ-opioid receptors were unchanged in cells expressing MOR/FLAG-CCR5 or FLAG-MOR alone, whereas the same treatment induced significant internalization of FLAG-CCR5 in MOR/FLAG-CCR5 or FLAG-CCR5 cells. These results indicate that pretreatment with DAMGO does not alter internalization of the chemokine CCR5 receptor and ,likewise, RANTES (CCL5) preincubation does not affect internalization of the μ-opioid receptor.

Table 4
EC_{50} and E_{max} values of RANTES (CCL5) and DAMGO in promoting [^{35}S]GTPγS binding following RANTES (CCL5) pretreatment

RANTES (CCL5)-pretreated	DAMGO			RANTES (CCL5)		
	EC_{50} (nM)	E_{max} (fmol/mg protein)	n	EC_{50} (nM)	E_{max} (fmol/mg protein)	n
Control	47.8 ± 15.3	305.3 ± 9.8	4	0.72 ± 0.26	298.0 ± 25.9	4
10 min	54.3 ± 8.9	209.5 ± 11.6	4	4.40 ± 0.93	192.5 ± 15.2	4
20 min	46.0 ± 2.6	217.7 ± 19.2	3	7.86 ± 1.96	227.0 ± 14.5	3
40 min	55.3 ± 1.2	240.0 ± 9.5	3	9.30 ± 2.50	220.0 ± 10.2	3

See Fig. 5 legend.

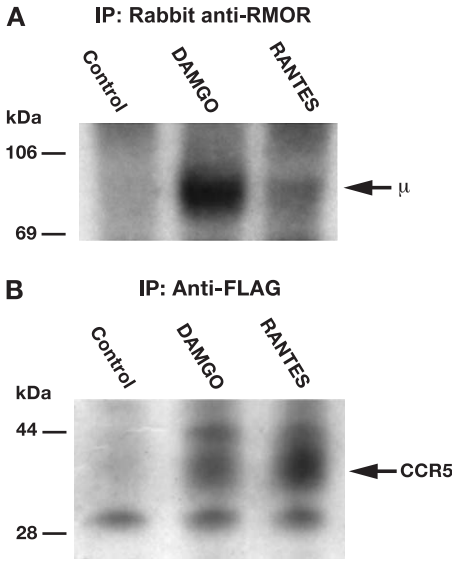

Fig. 6. Cross-phosphorylation of the μ-opioid and chemokine CCR5 receptors. CHO-MOR/FLAG-CCR5 cells were labeled with [^{32}P]phosphate and incubated with 1 μM DAMGO or 6.25 nM (50 ng/ml) RANTES (CCL5) at 37 °C for 10 min. Cells were lysed, solubilized, immunoprecipitated with (A) rabbit antiserum against a C-terminal domain peptide of the μ receptor or (B) rabbit polyclonal antibodies against the FLAG peptide, resolved by 10% SDS-PAGE, and subjected to autoradiography as described in Materials and Methods. The figures represent one of the five experiments performed with similar results. Longer incubation time up to 30 min yielded similar results.

4. Discussion

We have shown that the μ-opioid and chemokine CCR5 receptors coexpressed in CHO cells form heterodimers and cross-desensitize each other. Cross-desensitization is, at least in part, due to enhanced receptor phosphorylation and reduced receptor/G-protein. Heterodimerization of the two receptors may contribute to cross-desensitization.

4.1. Heterodimerization between the μ-opioid receptor and the chemokine receptor CCR5

The finding that HA-μ-opioid receptor and FLAG-CCR5 coimmunoprecipitated in cells coexpressing the two receptors, but not in a mixture of cells expressing individual receptors, demonstrates that the two receptors associate with each other when coexpressed. However, in Western blot analysis of immunoprecipitates, higher-molecular-weight species were not observed. In addition, phosphorylation studies revealed phosphorylated μ and chemokine CCR5 receptors at relative molecular weights similar to those reported previously for the individual receptors, but not any higher-molecular-weight bands. These results indicate that the interaction is sensitive to denaturing and/or reducing reagents, since the sample buffer for SDS-PAGE contains SDS and DTT, suggesting that interaction between the two receptors may involve noncovalent hydrophobic interactions and/or disulfide bond formation between the receptor proteins. Several dimerization interfaces have been proposed for GPCRs (Milligan, 2001; Devi, 2001; Angers et al., 2002); however, the actual mode of interactions between opioid and chemokine receptors requires further studies. Oligomerization of the μ-opioid receptor and CCR5 was reported in immune cells (Suzuki et al., 2002); however, the sharpness of the protein band and the molecular weight of the μ receptor differ from published reports (e.g., Zhang et al., 1996; El Kouhen et al., 1999). Different cell systems may play a role in the differences.

Agonist treatment had no effect on the level of heterodimerization between μ-opioid and chemokine CCR5 receptors as detected on Western blots (Fig. 2). Whether agonists affect homodimerization and heterodimerization of GPCRs depends on the receptor(s) involved (for reviews, see Milligan, 2001; Devi, 2001; Angers et al., 2002).

4.2. Chemotaxis

Although both DAMGO and RANTES (CCL5) induce chemotaxis, the concentration range in which RANTES (CCL5) is active is within a log unit, whereas that for DAMGO extends over three log units. The differences in the concentration ranges of an opioid vs. a chemokine that give a chemotactic response have been observed previously, and similar differences exist among the chemokines (e.g., Grimm et al., 1998a). For example, the chemotactic response of cells to CXCR4 ligands, such as stromal cell-derived factor-1α (CXCL12), occurs over a range of two to three orders of magnitude, while the optimal concentration of RANTES (CCL5) is much smaller. The capacity of chemoattractants to induce measurable chemotactic responses over different dose ranges is not understood. It may be due to differences in the relative capacity of these receptors to be desensitized. In those receptors that are particularly susceptible, this would result in reduced responsiveness or no responsiveness as the concentrations are increased. In addition, it is also possible that variability exists with respect to the specific G proteins that couple to a chemoattractant receptor (Arai and Charo, 1996). This may also contribute to the variability of responsiveness among GPCRs.

Our observation that the μ-opioid receptor agonist DAMGO promoted chemotaxis of CHO-MOR/FLAG-CCR5 cells is consistent with previous reports of opioids inducing chemotaxis in immune cells (Grimm et al., 1998a,b; Choi et al., 1999; Rogers et al., 2000; Miyagi et al., 2000). In addition, the findings that pretreatment with DAMGO reduced chemotaxis to RANTES (CCL5) and vice versa in CHO cells are in accord with previous studies in human peripheral blood monocytes and neutrophils and monkey leukocytes (Grimm et al., 1998a,b; Choi et al., 1999; Rogers et al., 2000; Miyagi et al., 2000). This indicates that CHO cells can be used as a model system to delineate possible mechanisms underlying cross-desensitization between these two receptors.

4.3. Receptor phosphorylation and receptor–G protein coupling

Pretreatment with DAMGO attenuated RANTES (CCL5)-induced [^{35}S]GTPγS binding; preincubation with RANTES (CCL5) likewise decreased DAMGO-promoted [^{35}S]GTPγS binding. Such a reduction in receptor–G protein coupling could be attributed to cross-phosphorylation of the μ-opioid and chemokine CCR5 receptors. Phosphorylation of unoccupied receptors by second messenger-dependent kinases activated by another receptor has been shown to contribute to cross-desensitization of chemoattractant GPCRs at the level of receptor–G protein coupling (for a review, see Ali et al., 1999). Zhang et al. (2003) recently showed that met-enkephalin inhibited both MIP-1α-mediated chemotaxis and Ca^{2+} flux of monocytes, and that Ca^{2+}-independent protein kinase C played an important role in heterologous desensitization.

DAMGO induced a more profound phosphorylation of the μ-opioid receptor than RANTES (CCL5), whereas RANTES (CCL5) increased phosphorylation of the CCR5 to a higher degree than DAMGO. This suggests that kinases involved in receptor phosphorylation and the residues phosphorylated in heterologous desensitization are likely to be different from those involved in homologous desensitization. GRK-mediated phosphorylation of agonist-occupied receptors has been shown to be involved in homologous desensitization of the μ-opioid receptor (Zhang et al., 1996) and the chemokine CCR5 receptor (Oppermann et al., 1999). The kinases contributing to the phosphorylation of the CCR5 and the μ-opioid receptor during cross-desensitization remain to be determined.

Differential receptor phosphorylation induced by DAMGO and RANTES (CCL5) may contribute to their differential effects upon the EC_{50} of the agonist-promoted [^{35}S]GTPγS binding in homologous and heterologous

desensitization. The EC_{50} value was increased only in homologous desensitization, but not in heterologous desensitization, although the E_{max} value was reduced in both. Consistent with this finding is the observation that pretreatment with DAMGO did not affect the affinity of RANTES (CCL5) for the CCR5, and incubation with RANTES (CCL5) did not change the affinity of DAMGO for the μ-opioid receptor. There are precedents in which receptor–G protein coupling is compromised with no changes in agonist affinity. For example, it was demonstrated that phosphorylation of the β_2-adrenoceptor by cAMP-dependent protein kinase, which induced heterologous desensitization, reduced receptor–G protein interaction, without affecting the binding affinity of isoproterenol to the receptor (Benovic et al., 1985). Since cAMP-dependent protein kinase phosphorylates the β_2-adrenoceptor at distinct sites from those for G protein-coupled receptor kinases (Lefkowitz et al., 1990), it is reasonable to assume that these differentially phosphorylated forms may adopt different conformations and hence exhibit different affinities for agonists. Whether this scheme is applicable to the cross-phosphorylated μ and CCR5 receptors requires further investigation. Our finding is different from a recent report by Zhang et al. (2003), who found that pretreatment of immune cells with met-enkephalin reduced the affinity of MIP-1α for the CCR1 receptor. Different chemokine receptors may be different.

Homologous desensitization and heterologous desensitization of the chemokine CCR5 receptor have differential impact on HIV entry. While homologous desensitization and internalization did not affect the activity of CCR5 as the HIV-1 coreceptor (Aramori et al., 1997), heterologous desensitization by activation of formyl peptide receptor or μ-opioid receptor impairs the HIV-1 coreceptor function of the chemokine CCR5 receptor (Shen et al., 2000; Szabo et al., in press).

Cross-phosphorylation of the receptors and reduction in receptor–G protein coupling may stem from heterodimerization of the receptors on the cell surface and sharing of common effector signal transducers such as G proteins. Both μ-opioid and chemokine CCR5 receptors have been reported to recruit PTX-sensitive G-proteins in CHO cells following agonist binding (Traynor and Nahorski, 1995; Zhao et al., 1998). However, it is not possible to define a causal relationship between heterodimerization and observed cross-desensitization as we have not found conditions that eliminate heterodimerization.

4.4. Homologous desensitization of the μ-opioid and chemokine CCR5 receptors

Following either DAMGO or RANTES (CCL5) treatment of CHO-MOR/FLAG-CCR5 cells, homologous desensitization was observed. This agrees with previous reports of homologous desensitization of the μ-opioid receptor (e.g., Zhang et al., 1996; El Kouhen et al., 1999) and the chemokine receptor CCR5 (Aramori et al., 1997; Zhao et al., 1998; Oppermann et al., 1999).

4.5. Discrepancy in the degree of cross-desensitization between chemotaxis and receptor–G protein coupling

Treatment of CHO-MOR/FLAG-CCR5 cells with RANTES (CCL5) abolished chemotactic response to DAMGO and vice versa. In contrast, preincubation with RANTES (CCL5) reduced the E_{max} value of DAMGO-promoted [^{35}S]GTPγS binding by ~30% without changing the EC_{50} value and vice versa. Thus, there is a difference in the degree of desensitization when chemotaxis and agonist-induced [^{35}S]GTPγS binding were used as the end points. The difference may be due to additional desensitization in the signal transduction pathway leading to chemotaxis, which requires further investigation.

The signal transduction pathways of GPCRs leading to chemotaxis have not been fully elucidated. Chemotaxis is a complex cellular response and may involve several different processes, such as cell polarization and shape change as well as cellular adhesion and migration (for a review, see Mellado et al., 2001). It was shown that stimulation of nonchemokine G_i-coupled receptors, but not G_s- or G_q-coupled receptors, promoted chemotaxis, and the release of free $G_{\beta\gamma}$ subunits of the G_i protein was required for chemotaxis (Neptune and Bourne, 1997; Arai et al., 1997). A number of cell type-dependent intracellular effectors such as arachidonic acid (Locati et al., 1994) and phosphatidylinositol 3-kinase (Haribabu et al., 1999) have been implicated, whereas others such as the p42/44 MAP kinase pathway and Ca^{2+} mobilization have been reported to be either necessary (Bacon et al., 1995; Groskopf et al., 1997) or not involved (Neptune and Bourne, 1997) in the regulation of cell migration via GPCRs. p38 MAP kinase activation and inhibition of adenylyl cyclase do not appear to be involved in chemokine-mediated chemotaxis (Neptune and Bourne, 1997).

RANTES (CCL5) and μ-opioid receptor agonists have been reported to induce tyrosine phosphorylation of the Src substrates p125FAK (focal adhesion kinase) and several cytoskeleton proteins (Bacon et al., 1996; Mangoura, 1997). Interaction of FAK with the cytoskeletal proteins, with their subsequent phosphorylation, has been reported to play a major role in cell polarization and migration (Clark and Brugge, 1995).

4.6. Receptor internalization

DAMGO caused internalization of the μ-opioid receptor but not CCR5; conversely, RANTES (CCL5) induced internalization of the CCR5, but not the μ-opioid receptor, in cells expressing both or alone. These findings suggested that the cross-desensitization between μ and CCR5 receptors may not be associated with receptor internalization in our system. These observations are similar to those of Szabo et

al. (in press), who found that activation of the μ-opioid receptor caused desensitization, but not internalization, of the chemokine CCR5 receptor in immune cells. Two possibilities may account for the lack of cointernalization of the two receptors. Although the receptors form heterodimers, they may be trafficked separately by the cell machinery. Heterodimers may dissociate before internalization and when some heterodimers are dissociated, others are formed as it is likely that there is an equilibrium between heterodimers and monomers of receptors. Another scenario is that since the percentage of receptors in heterodimers cannot be readily quantitated, the internalization results may be due primarily to the receptors not in heterodimers.

4.7. Concluding remarks

Cross-desensitization between the μ-opioid and chemokine CCR5 receptors, stably coexpressed in CHO cells, was demonstrated with both RANTES (CCL5) and DAMGO influencing in a reciprocal manner the chemotaxis, receptor phosphorylation, and [^{35}S]GTPγS binding of the other. Heterodimerization of the two receptors is likely to contribute to their cross-desensitization. Hence, our data suggest possible mechanisms for the inhibition of chemokine-induced directional migration of cells by which opiates and opioids may function in immunosuppression and inflammation, as well as for the desensitization of opioid-induced analgesia by RANTES (CCL5) administered into the periaqueductal gray. This cross-desensitization at the receptor and postreceptor levels may be utilized by the receptors to regulate the activity of the other and may contribute to alterations in intracellular effector pathways and subsequent impairment of immune function mediated by the other.

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