Selective Modulation of Wild Type Receptor Functions by Mutants of G-Protein-coupled Receptors

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Members of the G-protein-coupled receptor (GPCR) family are involved in most aspects of higher eukaryote biology, and mutations in their coding sequence have been linked to several diseases. In the present study, we report that mutant GPCR can affect the functional properties of the co-expressed wild type (WT) receptor. Mutants of the human platelet-activating factor receptor that fail to show any detectable ligand binding (N285I and K298stop) or coupling to a G-protein (D63N, D289A, and Y293A) were co-expressed with the WT receptor in Chinese hamster ovary and COS-7 cells. In this context, N285I and K298stop mutant receptors inhibited 3H-WEB2086 binding and surface expression. Co-transfection with D63N resulted in a constitutively active receptor phenotype. Platelet-activating factor-induced inositol phosphate production in cells transfected with a 1:1 ratio of WT:D63N was higher than with the WT cDNA alone but was abolished with a 1:3 ratio. We confirmed that these findings could be extended to other GPCRs by showing that co-expression of the WT C-C chemokine receptor 2b with a carboxyl-terminal deletion mutant (K311stop), resulted in a decreased affinity and responsiveness to MCP-1. A better understanding of this phenomenon could lead to important tools for the prevention or treatment of certain diseases.

Certain ligands can assume distinct functions in different tissues. For example, platelet-activating factor (PAF) is involved in embryogenesis as well as in modulation of a variety of functions of the immune and central nervous systems (1–3). With respect to PAF, for which the only known receptor is a member of the G-protein-coupled receptor (GPCR) family, the diversity of responses generated is likely a result of the receptor coupling to different signaling pathways in the target cells (3, 4). On the other hand, for other ligands such as adrenaline and dopamine, the cell can also determine the specificity of its response by the differential expression of receptor subtypes with distinct characteristics of binding, coupling, and desensitization (5). Subtypes of a given receptor can be derived from distinct genes or generated by alternative splicing. Divergence between receptor isoforms, as for the prostaglandin (EP) and the MCP-1 (CCR2) receptors, is most often limited to the carboxyl-terminal cytoplasmic tail, a region that is potentially involved in G-protein coupling, internalization, and down-regulation of the receptors (6, 7).

Alternative splicing can also lead to the formation of non-functional receptors or receptors with certain functions that are greatly modified (8, 9). Individually, these receptors are not involved in signaling, but some of them can show dominant-negative properties when co-expressed with a functional subtype. It has recently been demonstrated that the expression of a truncated isoform of the human gonadotropin-releasing hormone receptor could affect the extent of agonist-specific cellular response by inhibiting the cell surface expression of the functional isoform (10). A similar effect, that of confining the WT receptor intracellularly, was also reported for a deletion mutant (Δ32) of the CCR5 receptor, conferring a certain level of resistance to human immunodeficiency virus infection and delayed onset of acquired immune deficiency syndrome to heterozygous individuals (11). All the evidence of the dominant-negative effect of these truncated receptors points toward their capacity to form heterocomplexes with their respective WT functional receptors (8, 10, 11). The ability of an EP1 receptor isoform to inhibit signaling by EP1 as well as EP4 receptors suggests an association between the different receptor subtypes (8). The formation of heterocomplexes between different receptor subtypes may be an efficient mechanism to control the cellular response. For example, using immunoprecipitation and immunohistochemical studies, hetero-oligomerization between isoforms of the rat D3 dopaminergic receptor has been shown to occur in vivo (12).

Multimer formation has been shown or suggested for several other members of the GPCR family (13–20), including the human PAF receptor (hPAFR) (21, 22) and β2-adrenergic receptor (23), but he role of homo-oligomerization is still ill-defined. However, a study on the δ opioid receptor suggested that the transition between the oligomeric and monomeric forms of the receptor could be an important step in the internalization process (24). In addition, Herbert et al. (23) have suggested that oligomers constitute the active form of the β2-adrenergic receptor. They also demonstrated that exposure to an inverse agonist promoted the monomeric form. Thus, GPCRs could show similarities with the growth factor and cytokine receptors, whose activation depends on their transition from monomers to oligomers (25, 26).

The GPCRs are involved in a multitude of fundamental processes in higher eukaryotes such as development, homeostasis, and the transfer and integration of information (27). Several hereditary diseases have been linked to mutations within the coding regions of GPCRs (28–36). The dominance
phenomenon observed with some receptor isoforms and the evidence of a connection between oligomerization and activation raise the possibility of the existence of genetic diseases caused by GPCRs with dominant properties. In an attempt to verify whether some mutations could generate a dominant phenotype, two members of the GPCR family, the hPAFR and a splice variant (hCCR2b) of the human MCP-1 receptor, were co-expressed with mutant receptors incapable of undergoing an agonist-stimulated response or altered in other functions. Cell surface expression, ligand binding, and signaling characteristics were then evaluated. For the hPAFR, the WT receptor was co-transfected with the substitution mutants N285I (37), D289A (37), Y293A (38), and D63N (39) and the deletion mutant receptor K298stop (38). The N285I (37) and K298stop (38) mutant receptors do not show any specific binding of PAF (a phospholipid) or the antagonist WEB2086 (a benzoazepine). Substitutions of a highly conserved aspartate in the second transmembrane domain (D63N) or in the conserved motif D/N/PXXY of the seventh transmembrane domain (D289A) lead to a receptor with reduced internalization capacity but with an increased affinity for PAF (37–39). However, the substitution Y293A, in the same motif, did not significantly affect these properties (38). We also co-expressed the WT hCCR2b and a carboxyl terminus deletion mutant (K311stop), which did not show any detectable 125I-MCP-1 binding capacity, to evaluate whether our observations for the hPAFR could be extended to another member of the GPCR family.

MATERIALS AND METHODS

Construction of c-myc-tagged WT and Deletion Mutant Receptors—The WT hPAFR cDNA was a generous gift from Dr. Richard Ye (The Scripps Research Institute, La Jolla, CA). The mutant hPAFR-encoding cDNAs were constructed by polymerase chain reaction (PCR) and characterized as described previously (37–39). The WT hPAFR was subcloned into the pCMV (tagged with c-myc) and pJ3M (tagged with hemagglutinin) expression vectors (kindly provided by Dr. J. Chernoff, Fox Chase Cancer Center, Philadelphia, PA). hCCR2b WT encoding cDNA was amplified from MonoMac1 cell total RNA by reverse transcription-PCR using oligonucleotides CRKR2-FWD (5'-CCGGATCCCTGTCC-ACACCACCTTTATATTCAGA-3') and CRKR2-REV (5'-GGGTT-ACCTTATAAACCCGCGGACTCTTCGTCC-3'). The PCR product was digested with BamHI-Acc65I and subcloned into the pCMV expression vector. The carboxyl-terminal deletion mutant K311stop was constructed by PCR using the CRKR2-FWD primer and oligonucleotide 5'-CCGGATCCCTGTCC-ACACCACCTTTATATTCAGA-3'. The PCR product was subcloned into BamHI-Acc65I of pCMV. All constructions were sequenced (University of Calgary, Alberta, Canada).

Cell Culture and Transfections—CHO and COS-7 CHO cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum. Cells were plated in 30-mm dishes of Dulbecco's modified Eagle's medium high glucose and Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.), respectively, supplemented with 10% fetal bovine serum albumin and 20 mM LiCl for 5 min (hPAFR) or in Dulbecco’s modified Eagle’s medium containing only 10 mM LiCl for 10 min (hCCR2b). Cells were then stimulated for 1 min with the indicated concentrations of PAF or for 30 min with MCP-1. The reactions were terminated by the addition of perchloric acid. Inositol phosphates were determined by the addition of perchloric acid. Inositol phosphates were determined by the addition of perchloric acid. Inositol phosphates were determined by the addition of perchloric acid. Inositol phosphates were determined by the addition of perchloric acid. Inositol phosphates were determined by the addition of perchloric acid.

Flow Cytometry Studies—CHO and COS-7 cells co-transfected with c-myc-tagged hPAFR WT receptor and N285I or D63N constructions were harvested between 48 and 72 h after transfection, and 2.5 x 10^6 cells were subjected to flow cytometry analysis. For receptor expression studies (surface staining), labeling with anti-c-myc antibody (9E10 hybridoma supernatant) was used followed by a fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Bio/Can Scientific, Mississauga, Ontario, Canada), and labeling was as described previously (37). All measures were performed on a FACScan flow cytometer (Becton Dickinson).

Western Blot—COS-7 cells co-transfected with WT and pCDNA3 (WT) or WT and D63N (WT+D63N) in a 1:3 ratio were cultivated as described above and lysed at 4 °C for 30 min in a buffer containing 50 mM Tris-HCl (pH 7.5), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 2 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 1 mM Na2P04, 1 mM NaF, and 10 mg/mL of the protease inhibitors aprotinin and leupeptin. After the removal of debris by centrifugation, whole cell extracts were separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Proteins phosphorylated on tyrosine were revealed with anti-pTyr-01 and the ECL detection system (Amersham).

RESULTS

Receptor Distribution and Affinity for WEB2086 (Antagonist) in Cells Co-expressing WT and Mutant Receptors—Ligand binding was evaluated after co-transfection of WT hPAFR cDNA and different mutant constructs in CHO cells. 125I-WEB2086 binding characteristics were determined by competition with WEB2086. Affinity was not affected by co-expression of the D63N, D289A, or Y293A mutant receptors when compared with cells expressing only the WT receptor (Fig. 1A; Table I). In contrast, the N285I mutant receptor was efficient at preventing WEB2086 binding to either whole cells or membrane preparations (Fig. 1B). In Fig. 1B, results obtained for 125I-WEB2086 binding and cell surface expression (assessed by flow cytometry) were relative to those of cells expressing only the WT receptor (defined as 100%). Co-expression of the N285I mutant with the WT receptor also caused a significant decrease of cell surface expression of both receptors (Fig. 1B). Although WEB2086 binding was almost completely inhibited (3 ± 1%, residual binding) at a 1:1 ratio, the loss of surface expression of these receptors was only partial (23 ± 8%, residual expression). These results suggested the existence of receptors on the cell surface that are incapable of binding WEB2086. In addition, it is separated from the cells by centrifugation through Ficoll-Hypaque. The radioactivity contained in the cell pellet was counted on a LKB γ counter.

Inositol Phosphate Determination—COS-7 cells were co-transfected with WT and mutant receptor cDNA and, in the case of hCCR2b, also with the G-protein subunit Gαi (a generous gift from Dr. Melvin I. Simon (California Institute of Technology, Pasadena, CA)). Cells were labeled the following day for 18–24 h with myo-[3H]inositol (Amersham Corp.) at 3 μCi/mL in Dulbecco’s modified Eagle’s medium (high glucose, without insitol; Life Technologies, Inc.). After labeling, cells were washed and preincubated for 5 min in phosphate-buffered saline at 37 °C. The phosphate-buffered saline was removed, and cells were incubated in Dulbecco’s modified Eagle’s medium containing bovine serum albumin and 20 mM LiCl for 5 min (hPAFR) or in Dulbecco’s modified Eagle’s medium containing only 10 mM LiCl for 10 min (hCCR2b). Cells were then stimulated for 1 min with the indicated concentrations of PAF or for 30 min with MCP-1. The reactions were terminated by the addition of perchloric acid. Inositol phosphates were extracted and separated on Dowex AG1-X8 (Bio-Rad) columns. Total labeled inositol phosphates were then counted by liquid scintillation.

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is of interest to note that the cell surface expression of the co-expressed receptors was significantly lower (23 ± 8%) than that of the WT (defined as 100%) or of the N285I mutant receptor expressed alone (Fig. 1B). Confocal microscopy showed that the hemagglutinin-tagged WT and the c-myc-tagged N285I mutant receptors were efficiently expressed on the cell surface. The N285I mutant receptors, however, were not as evenly distributed and seemed to aggregate. When co-expressed, the distribution of these receptors changed to being localized intracellularly, for the most part (results not illustrated). The decreased cell surface expression was not due to an increased internalization rate of the co-transfected receptors because inhibitors of internalization failed to modify the distribution of WT+N285I complexes. No accumulation of receptors on the cell surface was observed by FACSScan analysis (WT+N285I untreated, 29 ± 12%; NH$_4$Cl, 31 ± 18%; sucrose, 31 ± 8%; dynamin K44A, 15 ± 8%; the percentages are in relation to WT expressed alone after undergoing the same treatment and set at 100%), although these inhibitors have been shown to efficiently block the internalization of hPAFR (38).

The influence of the K298stop mutant was comparable to N285I, and the extent of inhibition of expression and $^3$H-WEB2086 binding was dependent on the amount of co-transfected mutant cDNA, even to membrane preparations (data not shown). The similar effects of these two mutants suggest a comparable conformational change induced by the N285I substitution and the deletion of the last 44 amino acids of the carboxyl terminus (K298stop) in the seventh transmembrane domain.

Receptor Affinity for PAF in Cells Co-expressing WT and Mutant Receptors—We next characterized the binding and cellular response to PAF in cells co-expressing the same combination of constructs, as illustrated in Fig. 3. Affinity for the agonist was assessed by competition of $^3$H-WEB2086 binding by PAF (Fig. 2A). Transfection conditions were such that high receptor expression levels were attained, allowing for the measurement of binding properties independent of G-protein coupling (41). Binding isotherms were all uniphasic and were indicative of only one detectable class of binding sites, even in cells co-expressing receptors with different affinities (Fig. 2A). For instance, the D63N mutant has an ~6.7-fold higher affinity for PAF than the WT receptor when expressed individually (39), but after co-expression with the WT receptor, the apparent affinity was only ~1.9-fold higher than that of the WT alone (Table I). Similar results were obtained with D289A, which displays a ~2.8-fold higher affinity than the WT when expressed alone (37) and an apparent increase in affinity of 1.3-fold over the WT when these two receptors are co-expressed. These results suggest that a large proportion of the cell surface receptors, which bind WEB2086 and PAF, could be in an associated form of mutant/WT receptors. Co-expression of the WT and Y293A receptors resulted in an apparent affinity of 6.7-fold higher affinity after co-expression of WT and N285I or D289A receptors could not be done using $^3$H-WEB2086 because the level of specific binding was negligible. High levels of nonspecific $^3$H-PAF binding on membrane preparations also prevented an accurate evaluation of PAF binding (data not shown).

The numbers of cell surface receptors for the co-expressed WT+D289A and WT+Y293A were equivalent to the WT alone (Table I). When the WT was co-expressed with D63N in a 1:1 proportion, the cell surface receptor expression was slightly reduced (~25%) (Table I), but no further reduction was seen at a 1:3 proportion of WT:D63N in FACSScan analysis (data not shown).

The Effect of WT and Mutant hPAFR Co-expression on PAF-
None of the mutant receptors used in this study is capable of inducing IP production in response to PAF stimulation when expressed individually (37–39). We thus proceeded to look at the effect of co-expressing these mutants with the WT receptor on the PAF-induced IP response. COS-7 cells were co-transfected with the WT and either an equal (Fig. 2B) or three times greater amount of mutant receptor cDNA (Fig. 2C). IP accumulation was evaluated after a 1-min stimulation with PAF. Compared with COS-7 cells expressing only the WT receptor, the co-transfection of the Y293A or D289A mutants in a 1:1 proportion with the WT did not affect the dose-dependent response to PAF. EC$_{50}$ (median effective concentration) values could not be calculated because a plateau of response was not reached, even at the highest concentrations of PAF. Concentrations higher than 10$^{-6}$ M PAF have a detergent-like effect on the membrane and thus cannot be used. Co-transfection of N285I and WT receptor cDNA generated a slightly lower maximal IP response than that seen with the WT alone (Fig. 2B). A decrease (approximately 3-fold) was also observed when these two receptors were co-expressed in a 1:3 proportion of WT:N285I (Fig. 2C). It is of interest to note that the response levels (Fig. 2, B and C), cell surface expression, and $^{3}$H-WEB2086 binding (Fig. 1B) were all inversely proportional to the quantity of transfected N285I mutant cDNA. However, PAF-induced IP accumulation was inhibited by WEB2086 in the same manner in cells expressing the WT alone (IC$_{50}$ = 4.1 ± 0.5 μM) or in the presence of N285I (IC$_{50}$ = 4.5 ± 0.9 μM) mutant receptor (data not shown).

Co-expression of the D63N mutant with the WT receptor seemed to impart a constitutively active phenotype (Fig. 2D; Fig. 3, A and B). When co-transfected in a 1:3 proportion of WT:D63N, the baseline IP levels (308 ± 75%) were ~3-fold higher than those in cells expressing only the WT receptor (Fig. 2D). This increase represented about 85% of the level attained after stimulation of WT-transfected cells with 1 μM PAF. In these transfected cells (1:3 proportion of WT:D63N), there was no augmentation of IP levels after a stimulation, even with 1 μM PAF (Fig. 2C). However, when the proportion of WT:mutant
was 1:1, there was no inhibition of PAF-induced responsiveness; on the contrary, enhanced IP production could be observed (Fig. 2B), even with a baseline level (187 ± 26%) ~2-fold higher than the WT (defined as 100%) (Fig. 2D).

The co-transfection of WT+D63N also led to a constitutive increase of phosphotyrosine content as compared with WT-transfected cells (Fig. 3A). Western blotting indicated increased phosphorylation of bands at approximately 33, 40, and 43 kDa (Fig. 3B). There was also increased phosphotyrosine content in the region of higher molecular masses (89–205 kDa). When the blots were significantly underexposed, a major phosphorylated band was found at approximately 190 kDa in the WT+D63N lysate. The increase in cellular phosphotyrosine levels could also directly or indirectly participate in the basal IP production because genistein (a tyrosine kinase inhibitor) significantly decreased IP accumulation in the cells co-expressing the WT+D63N (1:3) complex (Fig. 3C).

Not all inactive mutant receptors were capable of affecting the apparent phenotype of cells expressing the WT hPAFR. The uncoupled mutant receptor Y293A had no effect on WT distribution, binding characteristics, or response. Other mutant receptors (C90A, C90S, C173A, and C173S) with altered cell surface expression and binding characteristics (41) did not modulate the WT receptor phenotype (data not shown).

The Effect of the Co-transfection of hCCR2b and K311stop on IP Production and MCP-1 Binding—To verify whether our observations with the hPAFR could apply to another GPCR, we performed experiments with the hCCR2b receptor. A modification in the binding characteristics and response was seen for this receptor when co-transfected with the deletion mutant receptor K311stop (Fig. 4A). Cells transfected with this mutant receptor cDNA (and Gαq) did not show any response to MCP-1 or specific 125I-MCP-1 binding, even to membrane preparations. However, by co-expressing this mutant with the WT receptor, a shift in affinity from a Kd of 320 ± 20 to 610 ± 35 pM was observed (Fig. 4A). It also had a significant inhibitory effect on the response to MCP-1, as measured by IP production (Fig. 4B). This inhibition may be due to the decrease in affinity for the agonist induced by the co-expression of the mutant and WT receptors.

DISCUSSION

This study demonstrates the possibility of using the co-expression of certain point substitution or deletion mutants to modulate the response and expression of the WT GPCR. The resultant response, after co-transfection, is not necessarily the sum of the individual characteristics of each receptor because certain mutants induced a dominant-negative effect such as the inhibition of surface expression (N285I and K298stop) or created a new phenotype such as constitutive activation (D63N). Selective modulation of binding characteristics for the agonist (D289A, D63N, and K311stop) and decreased cell surface expression (N285I and K298stop) have also been observed.

It is of interest to note that the cell surface expression of the co-expressed receptors was significantly lower (23 ± 8%) than that of the WT (defined as 100%) or of the N285I (65 ± 15%) mutant receptor expressed alone. In addition, confocal microscopy indicated that co-expression of the WT and mutant receptors leads to receptor redistribution. This effect was not due to an increase in constitutive internalization because inhibitors of hPAFR internalization did not alter the cell surface expression of the mutant:WT receptors. Rather, the receptor redistribution may be the result of faulty membrane targeting. Interestingly, at a 1:1 ratio of WT:N285I, there was only a partial loss of surface receptor protein expression (23 ± 8%, residual expression), but WEB2086 binding was almost completely inhibited (3 ± 1%, residual binding). These results suggest the
existence of cell surface receptors whose capacity of binding WEB2086 had been altered by the co-expression of the N285I mutant. This particular population does not seem to participate in PAF-induced IP production, because this response is sensitive to WEB2086 inhibition in the same manner as in cells expressing only the WT receptor (results not shown). The lower PAF-induced maximal response observed with these co-transfected cells could also be attributed to the substantial loss of PAF-induced maximal response observed with these co-transfected cells. The lower PAF-induced maximal response observed with these co-transfected cells could also be attributed to the substantial loss of surface expression after co-expression of two distinct receptors such as the affinity for ligand, the level of basal activity, and the extent of cell surface expression, presumably by the occurrence of hetero-oligomerization. This raises the possibility that naturally occurring splice variants, such as C22a and C22b, with distinct coupling, distribution, and desensitization characteristics could associate in a hetero-oligomeric complex and permit a subtle control of the ligand-induced response by modulating the ratio of the two receptors. Indeed, splice variants of the gonadotropin-releasing hormone receptor and the EP1 subtype of prostaglandin E2 receptor attenuate the action of the specific ligands (8, 10). This is also the case for the carboxyl-terminal deletion mutant of C22b whose presence modulates the ligand affinity and responsiveness to MCP-1. This study showed that a simple substitution of an amino acid of a GPCR could lead to a dominant-negative phenotype. We also demonstrate that co-expression of an uncleaved mutant (D63N) with the WT receptor could lead to an apparent constitutively active phenotype highly dependent on receptor:mutant ratio. Interestingly, even though D63N does not seem

form complexes that may be composed of two or more receptor molecules and that these complexes define the properties of the receptor. This is supported by: 1) the fact that binding curves were all uniphasic for co-expressed receptors with different affinities, 2) a shift in affinity for MCP-1 was observed with the co-expression of the hCCR2 and K311stop mutant, and 3) the efficiency of the N285I mutant in inhibiting WEB2086 binding to hPAFR. Our hypothesis is also supported by the results of other groups, which show not only dimeric receptors but also complexes of much higher molecular weight on SDS-polyacrylamide gel electrophoresis for the hPAFR (20, 22) and other members of the GPCRs (12–14, 42). One must also consider that these results could be the result of a greater predisposition toward the formation of heterocomplexes than homocomplexes. This type of interaction has been reported for the dopamine D3 receptor and a nonfunctional splice variant (12). Our data also showed that different ratios of D63N:WT modulate the phenotype of the complex. A 1:3 WT:mutant ratio drives the complex toward a more constitutively active conformation than a 1:1 ratio and abolishes responsiveness to PAF.

The concept of the formation of a hetero-oligomeric complex was first introduced by the demonstration of the functional rescue of receptor properties such as agonist binding, signaling, and cell surface expression after co-expression of two distinct nonfunctional mutants or chimeric receptors (15–18). Recently, it has been shown that hetero-complexes formed between the CCR5 receptor and a deletion mutant, CCR5Δ32, retained the WT in the cellular compartment and thus reduced cell surface expression of the functional receptor (11). Similar results have also been obtained with a splice variant of the human gonadotropin-releasing hormone receptor (10). The fact that some mutant receptors, which are themselves only expressed intracellularly, inhibit the expression of the WT on the cell surface suggests that the oligomerization takes place very early after protein synthesis, possibly in the endoplasmic reticulum, where the mutants seem trapped. Adequate folding seems to be necessary for the association of the receptors because mutants of the hPAFR, which are thought to lack disulfide bonds (43), did not influence the WT phenotype when co-expressed in CHO cells.2

Our results expand these observations by demonstrating that co-expression of receptors altered in certain functions could also be used to modify specific characteristics of WT receptors such as the affinity for ligand, the level of basal activity, and the extent of cell surface expression, presumably by the occurrence of hetero-oligomerization. This raises the possibility that naturally occurring splice variants, such as C22a and C22b, with distinct coupling, distribution, and desensitization characteristics could associate in a hetero-oligomeric complex and permit a subtle control of the ligand-induced response by modulating the ratio of the two receptors. Indeed, splice variants of the gonadotropin-releasing hormone receptor and the EP1 subtype of prostaglandin E2 receptor attenuate the action of the specific ligands (8, 10). This is also the case for the carboxyl-terminal deletion mutant of CCR2b whose presence modulates the ligand affinity and responsiveness to MCP-1.

2 C. Le Gouill, J.-L. Parent, C.-A. Caron, R. Gaudreau, L. Volkov, M. Rola-Pleszczynski, and J. Stašková, unpublished observations.

FIG. 4. Characterization of cells co-expressing WT and deletion mutant cDNAs of hCCR2b. A, competition binding isotherms of 125I-MCP-1 by MCP-1. 125I-MCP-1 binding was measured as indicated under “Materials and Methods” on CHO cells that were transiently co-transfected with the WT (1 μg) and K311stop mutant receptor encoding cDNAs (1 μg). B, inositol phosphate accumulation in response to graded concentrations of MCP-1 (0.1, 1, 5, 20, and 80 nM). Total IPs were measured after a 30-min stimulation with the indicated MCP-1 concentrations of COS-7 cells co-transfected with 150 ng of the G-protein subunit Gα1c cDNA and either hCCR2b WT or K311stop receptor cDNA, alone (150 ng) or in combination (1:1 ratio (150 ng WT:150 ng K311stop) or 1:3 ratio (150 ng WT:450 ng K311stop)). IP quantification was as described under “Materials and Methods.” The results are representative of three independent experiments done in duplicate.
capable of G-protein coupling, it demonstrates a higher affinity for its ligand, a characteristic that it shares with constitutively active mutants. The co-expression of nonfunctional mutants, chimeric receptors, and a recent study by Hebert et al. (44) have shown that rescue of receptor function is achievable through oligomerization (15–18, 44). It is therefore conceivable that oligomerization between the WT and D63N receptors may result in a complex that possesses both the capacity for G-protein coupling and certain characteristics of constitutively active receptors. The fact that the 1:3 WT:D63N was unresponsive to PAF could be due to the fact that all receptor complexes are already in an active conformation. This constitutive activation would also result in a certain quantity of receptors being desensitized and internalized, which may also explain the fact that the levels of basal IP accumulation (1:3 WT:D63N) are lower than if one stimulates a population of WT receptors, of which none are desensitized. The combination of WT and D63N also resulted in an increase in tyrosine phosphoproteins as which none are desensitized. The co-expression of nonfunctional mutants, chimeric receptors, and a recent study by Hebert et al. (44) have shown that rescue of receptor function is achievable for its ligand, a characteristic that it shares with constitutively active mutants.

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