Rescue of Functional Interactions between the $\alpha_{2A}$-Adrenoreceptor and Acylation-resistant Forms of $G_{11}\alpha$ by Expressing the Proteins from Chimeric Open Reading Frames*

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Co-expression of the $\alpha_{2A}$-adrenoreceptor with a pertussis toxin-resistant (C351G), but not with an also palmitoylation-resistant (C3S/C351G), form of the $\alpha$ subunit of $G_{11}$ resulted in agonist-induced, pertussis toxin-independent, GTP hydrolysis. Construction and expression of a chimeric fusion protein between the receptor and C351G $G_{11}\alpha$ generated a membrane protein in which the $G$ protein element was activated by receptor agonist. An equivalent fusion protein containing C3S/C351G $G_{11}\alpha$ rescued the ability of receptor agonist to activate this mutant. Fusion proteins of a palmitoylation-resistant (C442A) $\alpha_{2A}$-adrenoreceptor and either C351G or C3S/C351G $G_{11}\alpha$ also responded effectively to agonist. Myristoylation resistant (G2A/C351G) and combined acylation-resistant (G2A/C3S/C351G) mutants of $G_{11}\alpha$ are cytosolic proteins. Expression of these as chimeric $\alpha_{2A}$-adrenoreceptor-$G_{11}$ protein fusions restored membrane localization and activation of the $G$ protein by receptor agonist. These studies demonstrate the general utility of generating chimeric fusion proteins to examine receptor regulation of $G$ protein function and that the lack of functional activation of acylation-negative $G$ proteins by a co-expressed receptor is related to deficiencies in cellular targeting and location rather than an inherent incapacity to produce appropriate protein-protein interactions and signal transmission.

A major mechanism for signal transduction across the plasma membrane involves seven transmembrane element $G$ protein-coupled receptors (GPCRs) and their activation of members of the family of $\alpha\beta\gamma$ heterotrimeric guanine nucleotide binding proteins ($G$ proteins) (1–2). Unlike the GPCRs, none of the individual $G$ protein subunits contain transmembrane-spanning elements although the proteins are membrane-associated. In the case of the $\beta\gamma$ complex, post-translational prenylation by the C15 farnesyl and C20 geranylgeranyl groups at a cysteine residue close to the C-terminal tail of the $\gamma$ subunit followed by protein trimming and carboxymethyla-

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† The abbreviations used are: GPCR, G protein-coupled receptor; $G$ protein, guanine nucleotide binding protein; UK14304, 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine; ORF, open reading frame; bp, base pair(s).

EXPERIMENTAL PROCEDURES

Materials—All materials for tissue culture were supplied by Life Technologies, Inc. (Paisley, Strathclyde, Scotland). [3H]RS-79948–197 (90 Ci/mmol) was purchased from Amersham International. [32P]GTP (30 Ci/mmol) was obtained from NEN Life Science Products. Pertussis toxin (240 $\mu$g/ml) was purchased from Speywood. All other chemicals were from Sigma or Fisons plc and were of the highest purity available.
Oligonucleotides were synthesized on a Millipore Expedite Nucleic Acids Synthesis System.

Construction of the α₂A-Adrenoreceptor-C351G G i1α Fusion Constructs—A pertussis toxin-resistant C351G form of G i1α was generated (20) and linked to the porcine α₂A-adenoreceptor (22). To do so, the ORF of the α₂A-adrenoreceptor was amplified by polymerase chain reaction using the oligonucleotides: sense, 5’-TGGTACCATGTATGC-TCTACGAGTTC-3’ and antisense, 5’-AAAGTTGCTATGGGATC-GCTTTCTGCTGCCCCACGGC-3’ (restriction sites for KpnI, EcoRI, and NcoI are underlined). The polymerase chain reaction-amplified fragment was digested with KpnI and EcoRI and ligated to pBluescript (Stratagene) through these restriction sites. Introduction of the NcoI site at the 3’-end of the ORF resulted in the C-terminal amino acid of the receptor being altered from Val to Ala and removal of the stop codon. The rat C351G G i1α cDNA contains two NcoI sites, one straddling the ATG start codon and the other 268 bp downstream from this. This 268-bp fragment was removed from C351G G i1α in pBluescript by digestion with NcoI, and the remaining C351G G i1α pBluescript cDNA was religated. The shortened cDNA was excised from pBluescript with EcoRI and cloned into the EcoRI site of the α₂A-adrenoreceptor in pBluescript, adjacent to the 3’-end of the receptor ORF. The 268-bp fragment was then inserted between the NcoI sites at the 3’-end of the α₂A-adrenoreceptor ORF and at the 5’-end of the C351G G i1α ORF. This resulted in production of an in-frame construct whereby the 3’-end of the C351G G i1α ORF was exactly adjacent to the 5’-end of the C351G G i1α ORF. The full fusion construct was then excised from pBluescript with KpnI and EcoRI and ligated into the eukaryotic expression vector pCDNA3. The same strategy was used to generate the various acylation-deficient chimeras.

Cell Culture and Transfection—COS-7 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% (v/v) fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were seeded in 60-mm culture dishes and grown to 60–80% confluency (18–24 h) prior to transfection with pcDNA3 containing the relevant cDNA species using LipofectAMINE reagent (Life Technologies, Inc.) and incubated at room temperature for 30 min prior to the addition of 1.8 ml of Opti-MEM. COS-7 cells were exposed to the DNA/LipofectAMINE mixture for 5 h. 2 ml of 20% (v/v) fetal calf serum in Dulbecco’s modified Eagle’s medium were then added to the cells. Cells were harvested 48 h after transfection. In all studies, the cells were treated with pertussis toxin (25 ng/ml) 24 h prior to cell harvest to eliminate possible interactions of the fusion proteins with endogenously expressed G i1α family G proteins (19–20).

Preparation of Membranes—Plasma membrane-containing P2 particulate fractions were prepared from cell pastes that had been stored at −80 °C following harvest. Cell pellets were resuspended in 0.5 ml of 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5 (buffer A), and rupture of the cells was achieved with 50 strokes of a hand-held Teflon on-glass homogenizer followed by passage (10 times) through a 25-gauge needle. Cell lysates were centrifuged at 1900 × g for 10 min in a Beckman TJ-6 centrifuge to pellet the nuclei, and unbroken cells and P2 particulate fractions were then recovered by centrifugation of the supernatant at 200,000 × g for 30 min in a Beckman TL 100 bench-top ultra-centrifuge using a Beckman TLA 100.2 rotor. P2 particulate fractions were resuspended in buffer A and stored at −80 °C until required.

1HIRS-79948–197 Binding Studies—Binding assays were initiated by the addition of 2–4 µg of protein to an assay buffer (10 mM Tris-HCl, 50 mM sucrose, 20 mM MgCl₂, pH 7.5) containing 1HIRS-79948–197 (23) (1 nM). Kᵢ for this ligand at the fusion protein derived from the wild-type receptor and G protein = 0.35 nM (20). Nonspecific binding was determined in the presence of 100 µM iodoazoxan. Reactions were incubated at 30 °C for 45 min, and bound ligand was separated from free by vacuum filtration through GF/C filters. The filters were washed 3 times with 5 ml of assay buffer, and bound ligand was estimated by liquid scintillation spectrometry.

Immunological Studies—Antiserum IIC (24) was produced in a New Zealand White rabbit using a conjugate of a synthetic peptide corresponding to amino acids 160–169 of the G i1α subunit and keyhole limpet hemocyanin (Calbiochem) as antigen. The specificity of this antiserum for G i1α has been demonstrated previously (24). Membrane samples were resolved by SDS-polyacrylamide gel electrophoresis using 10% (w/v) acrylamide gels containing 6 M urea overnight at 100 V. Proteins were subsequently transferred to nitrocellulose (Schleicher & Schuell), probed with relevant antiserum, and visualized as described (25).

High Affinity GTPase Assays—High affinity GTPase assays were performed essentially as described previously (26) using [γ-32P]GTP (0.5 µM, 60,000 cpm) and varying concentrations of UK14304 (up to 10 µM). Nonspecific GTPase was assessed by parallel assays containing 100 µM GDP.

RESULTS

Expression of the porcine α₂A-adrenoreceptor (22) in COS-7 cells allows agonist activation of endogenously expressed G proteins as measured by the capacity of the α₂A-adrenoreceptor agonist UK14304 to stimulate high affinity GTPase activity in membranes of these cells (20). Pertussis toxin treatment prior to cell harvest attenuates this effect, demonstrating it to reflect activation of G i-family G proteins (20). A C351G mutation was introduced into G i1α to render this protein insensitive to pertussis toxin (20). Co-expression of this modified G protein with the α₂A-adrenoreceptor in COS-7 cells, followed by treatment of the cells with pertussis toxin, now resulted in a robust stimulation of high affinity GTPase activity upon addition of UK14304 (Fig. 1). A CS mutation of G i1α prevents this polypeptide from acting as a substrate for post-translational myristoylation (9–10). Co-expression of C3S/C351G G i1α and the α₂A-adrenoreceptor failed to allow UK14304 stimulation of the GTPase activity of this mutated G protein (Fig. 1) although high levels of the G protein can be expressed in the membrane preparation (21). A G2A mutation of G i1α prevents co-translational myristoylation of this protein (5–6), and the combination of G2A and CS mutations totally eliminates acylation of the expressed polypeptide (9) and prevents membrane attachment. Co-expression of these forms of G i1α, which also contained the C351G mutation with the α₂A-adrenoreceptor, did not result in UK14304 stimulation of high affinity GTPase activity (21) as they were virtually entirely cytosolic (data not shown, but see Ref. 21).

The open reading frames of each of C351G G i1α, C3S/C351G G i1α, G2A/C351G G i1α, and G2A/C3S/C351G G i1α were fused in-frame to the wild-type α₂A-adrenoreceptor cDNA to create chimeric fusion proteins that contained the entire G protein α subunit sequence downstream of the receptor. This process introduced a single substitution of the receptor C-terminal Val by Ala and the maintained presence in the fusion proteins of the initiator Met of G i1α (Fig. 2).
Expression of each of these α2A-adrenoceptor-C351G Gi1α constructs in COS-7 cells resulted in high levels of membrane expression of the receptor binding site (some 15–25 pmol/mg protein in individual transfections) as measured by the specific binding of the highly selective and high affinity α2-antagonist [3H]RS-79948–197 (23). There were no differences in levels of expression of the individual fusion proteins in concurrently performed transfections (data not shown, but see Fig. 5). Immunoblotting membranes and cytosolic fractions derived from these transfections with the Gi1α specific antiserum I1C (24) resulted in identification of low levels of the endogenously expressed Gi1α specific antiseraum I1C (24) expected size for the fusion proteins, a polypeptide of some 100 kDa was detected.

Membranes of cells expressing the α2A-adrenoceptor-C351G Gi1α fusion protein were able to support concentration-dependent stimulation of pertussis toxin-insensitive high affinity GTPase activity upon addition of UK14304 (EC₅₀ = 2.3 ± 0.3 × 10⁻⁷ M) (Fig. 4A). In contrast to the lack of functional interactions between co-expressed but independent α2A-adrenoceptor and C35/C351G Gi1α (Fig. 1), expression of the fusion protein containing the C35 mutation also resulted in agonist-mediated stimulation of high affinity GTPase activity (EC₅₀ = 4.4 ± 1.1 × 10⁻⁷ M) (Fig. 4A). This was also true for the two fusion proteins containing the G2A mutation in the G protein (Fig. 4A) and again EC₅₀ values for UK14304 (G2A/C351G Gi1α = 3.5 ± 0.8 × 10⁻⁷ M, G2A/C35/C351G Gi1α = 3.2 ± 0.9 × 10⁻⁷ M) (mean ± S.E., n = 3 in each case) were not different.

The porcine α2A-adrenoceptor has a cysteine residue 9 amino acids from the C terminus that has been shown to act as an acceptor for post-translational palmitoylation (27). Although a C442A mutation of the receptor has been reported not to interfere with G protein activation (27), we considered whether potential palmitoylation of this cysteine might alter agonist regulation of the fusion proteins as palmitoylation is believed to provide an extra site of membrane anchorage and generate a “fourth intracellular loop” in the receptor (11). Furthermore, the C-terminal tail of the α2A-adrenoceptor is only some 20 amino acids long, which is relatively short within the family of G protein-coupled receptors, and we wished to consider if the extra flexibility following removal of the site of palmitoylation might influence regulation of fusion protein activation by agonist. Fusion proteins containing the pertussis toxin-insensitive C351G Gi1α in concert with G2A, C3S, and...
G2A/C3S mutations were constructed with the C442A \( \alpha_{2A} \)-adrenoreceptor (Fig. 2) and expressed in COS-7 cells. Each of these constructs was expressed to a similar level as those containing the wild-type receptor (Fig. 3), and each was able to stimulate pertussis toxin-insensitive high affinity GTPase activity in response to UK14304 to similar extents and with similar EC\(_{50}\) values (2.2–2.8 \( \times 10^{-7} \) M in individual transfections) as the fusion proteins containing the wild-type receptor sequence (Fig. 4B).

Co-expression of the \( \beta_2 \gamma_2 \) complex with the wild-type \( \alpha_{2A} \)-adrenoreceptor-C351G \( G_i \alpha \) fusion protein resulted in substantially greater maximal stimulation of GTPase activity by UK14304 than achieved without excess \( \beta_2 \gamma_2 \) (Fig. 5A). This did not reflect increased levels of expression of the fusion protein (Fig. 5B) or alterations in the EC\(_{50}\) for UK14304 (data not shown but see (19)). Additional expression of \( \beta_1 \gamma_2 \) also increased the maximal GTPase activity in response to UK14304 of each of the G2A/C351G, C3S/C351G, and G2A/C3S/C351G forms of \( G_i \alpha \) constrained in fusion proteins with the wild-type \( \alpha_{2A} \)-adrenoreceptor (Fig. 5A), consistent with interaction of the \( \beta_1 \gamma_2 \) complex with all of the individual fusion proteins. This is despite the known role of the N-terminal region of the G protein \( \alpha \) subunit, which is constrained in the fusion constructs, in \( \beta \gamma \) binding (28–29).

**DISCUSSION**

Acylation of G protein \( \alpha \) subunits plays a key role in the membrane targeting and association of these proteins (3–4, 11). Furthermore, prevention of myristoylation of the \( \alpha \) subunit of \( G_i \)-like G proteins reduces their affinity of interaction with the \( \beta \gamma \) complex and can render them cytoplasmic (5–6). Prevention of palmitoylation also limits membrane association and has been reported to limit or prevent interactions with receptors (7, 9, 12, 14). However, it has been unclear whether poor activation of acylation-defective G protein \( \alpha \) subunits by receptors is simply a problem associated with the lack of appropriate targeting and thus proximity to a receptor or is inherently due to the acylation status of the G protein.

In the current study, we have taken a highly novel approach to address this question. This has involved the construction of chimeric fusion proteins between the \( \alpha_{2A} \)-adrenoreceptor and the \( \alpha \) subunit of the G protein \( G_i \). To do so involved the apparently simplistic expedient of linking the N terminus of the G protein directly to the C terminus of the receptor. The strategy used resulted in a minimal alteration to the sequence of the protein in the region of fusion. Indeed, only the C-terminal amino acid of the receptor was altered (Val to Ala), and the initiator Met of the G protein, which would normally be removed, remained in the sequence of the new protein (see Fig. 2). Only a single previous example has examined the potential for signal transduction following expression of a GPCR fused to its cognate G protein \( \alpha \) subunit. In the case of the \( \beta_2 \)-adrenoreceptor-\( G_i \alpha \) chimera, addition of agonist was able to cause activation of adenyl cyclase following expression of the fusion protein in a cell line that genetically lacks endogenous \( G_i \alpha \) (18).

As well as using the wild-type receptor and G protein, fusion chimeras were generated between the wild-type \( \alpha_{2A} \)-adrenoreceptor and mutant forms of \( G_i \alpha \) in which the sites that are normally palmitoylated and myristoylated in the G protein were modified. Expression of each of these constructs could be detected in the membrane fraction following transient transfection of each of these in COS-7 cells. This was achieved both in binding assays using the highly selective and high affinity \( \alpha_{2A} \)-adrenoreceptor antagonist \([\text{H}]\text{RS-79948–197}\) to detect the ligand binding site of the receptor (Fig. 5) and by immunoblotting cytosolic and membrane fractions of these cells with the specific \( G_i \alpha \) antisemur H1C (24) (Fig. 3). \( G_i \alpha \) is expressed endogenously in COS-7 cells at low levels and could be detected as a 41-kDa polypeptide by this antiserum in membranes from both mock and positively transfected cells. In contrast, membranes of positively transfected but not mock-transfected cells displayed the presence of an H1C reactive polypeptide of some 100 kDa, which corresponded to the chimeric fusion proteins (Fig. 3). Similar levels of the fusion proteins were expressed whether the G protein element of the fusion protein was wild type at the N terminus or contained G2A, C3S, or both mutations (Fig. 3), and none of these immunoreactive proteins were detected in the cytosolic fractions.

As with many GPCRs (11), the \( \alpha_{2A} \)-adrenoreceptor is also a target for post-translational palmitoylation, at Cys442 within the C-terminal tail. Because this acylation is proposed to create
a “fourth intracellular loop” in the receptor structure and because this receptor has only a short C-terminal tail, we also created chimeric fusion proteins between a C442A mutant of the α2A-adrenoreceptor and the various forms of G_{i,α} detailed above. Although previous studies have indicated this mutation in the receptor not to interfere with agonist-mediated G protein activation, we wished to assess whether potential greater flexibility provided by a longer “linker” between the seventh transmembrane element of the receptor and the N terminus of the G protein α subunit would affect agonist activation of the G protein. All of the C442A-α2A-adrenoreceptor-G_{i,α} fusions were also expressed and to similar levels as the versions that included the wild-type receptor (Fig. 3).

As an aid to subsequent analysis, we generated all of the fusion proteins using a form of G_{i,α} in which Cys-351, which is the normal target for pertussis toxin-catalyzed ADP-ribosylation, was substituted by Gly. We have previously demonstrated that C351G G_{i,α} is not a substrate for pertussis toxin-catalyzed ADP-ribosylation (20). It can, however, be activated by the α2A-adrenoreceptor, with the only clear difference, compared with the wild-type G protein, being that some 10–15-fold higher concentrations of agonist are required to produce the same degree of stimulation (20). This is presumably a reflection of the alteration in conformation of the C terminus of the G protein, an element known to be a key receptor contact site (30). Such mutations, however, allow receptor regulation of the mutated G protein to be studied in isolation following pertussis toxin-treatment of cells to eliminate potential functional contacts between the receptor and the endogenously expressed G_{i,α}-family G proteins (20, 31, 32). As such, all of the functional experiments herein were performed following pertussis toxin treatment of the cells for times and with amounts of toxin that cause modification of all of the endogenous G_{i,α}-like G proteins (20).

All of the expressed chimeric fusion proteins were able to stimulate high affinity pertussis toxin-insensitive GTPase activity in membranes of the transfected COS-7 cells upon addition of the α2A-adrenoreceptor agonist UK14304. Agonist stimulation of high affinity GTPase activity is a classical assay to measure both GDP-GTP exchange and then subsequent GTP hydrolysis by a G protein α subunit (25–26). As such, these results demonstrate both that the G protein α subunit is folded appropriately to exchange guanine nucleotide and to act as an enzyme and that information, presumably mediated via conformational change, can be transmitted from the binding of agonist to the receptor on to the G protein. These data thus further demonstrate the general utility of generating chimeric fusion proteins to examine receptor regulation of G protein function (18–19).

G2A and G2A/C3S mutants of G_{i,α} are essentially completely cytosolic (9), and thus it is not surprising that they are not activated by receptor agonists (21). Physical proximity, as guaranteed from the fusion protein approach, showed that the lack of functional activation of such acylation negative mutants of G proteins by a co-expressed receptor is related to deficiencies in cellular targeting and location rather than an inherent incapacity to produce appropriate protein-protein interactions for signal transmission. As the fusion chimeras containing the G2A and C3S mutants required similar concentrations of UK14304 to cause half-maximal effects, this also argues that acylation is not inherently required to produce effective protein-protein contacts between the receptor and G protein α subunit but rather is to position the G protein appropriately. It was noted, however, that the fusion constructs containing both the G2A and C3S mutations in G_{i,α} displayed somewhat lower GTPase activity in response to maximally effective concentrations of UK14304 rather than the other constructs (Fig. 4). The reason for this requires further investigation. The C3S mutation of G_{i,α} results in expression of a protein that is partially cytoplasmic but partially membrane-associated (9). As such, by increasing levels of expression of C351G G_{i,α}, it is possible to obtain levels of this mutant at the membrane as high or higher than that following expression of wild-type G_{i,α} (21). Despite this, the independently expressed α2A-adrenoreceptor is unable to cause significant activation of C351G G_{i,α} (Fig. 1). The reason for this remains to be resolved as the fusion proteins containing C351G G_{i,α} were as effectively stimulated by UK14304 as any of the others (Fig. 4).

Given the nature of the physical linkage between the receptor and G protein in the fusion proteins, the knowledge that the N terminus of the G protein α subunit plays a central role in interaction with the βγ complex (28–29), the concept that the βγ complex may play a key role in receptor interactions with the α subunit (33–35), and the understanding that G protein α subunit acylation is important in interactions with the βγ complex, it was of considerable interest to observe that co-expression of excess β2γ2 along with any of the α2A-adrenoreceptor-G_{i,α} fusion proteins resulted in greater maximal UK14304 stimulation of GTPase activity (Fig. 5). A trivial explanation for this observation based on higher steady-state levels of expression of the fusion protein in the presence of excess βγ was eliminated by performing [3H]-antagonist binding studies (Fig. 5). These results imply interaction of the fusion protein with the βγ complex, but understanding the details of this will require further study. It is of interest in this regard to note, however, that Taylor et al. (36) have previously indicated a role for βγ in receptor stimulation of the GTPase activity of the α2A-adrenoreceptor.

Overall these studies demonstrate the receptor-G protein fusion approach to be a novel and useful means to study receptor-G protein interactions and indicate a key role for G protein acylation in cellular targeting but not intrinsically in transmission of information between receptors and G proteins.

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REFERENCES

1. Gilman, A. G. (1987) Annu. Rev. Biochem. 56, 615–649
2. Bourne, H. R., Sanders, D. A., and McCormick, F. (1991) Nature 348, 125–132
3. Casey, P. J. (1995) Science 268, 221–225
4. Wedegaertner, P. B., Wilson, P. T., and Bourne, H. R. (1995) J. Biol. Chem. 270, 503–506
5. Munby, S. M., Heuckeroth, R. O., Gordon, J. I., and Gilman, A. G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 728–732
6. Jones, T. L. Z., Simonds, W. F., Merendino, J. J., Brann, M. R., and Spiegel, A. M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 568–572
7. Parenti, M., Viganò, M. A., Newman, C. H. M., Milligan, G., and Magee, A. I. (1990) Biochem. J. 269, 349–353
8. Geppert, P., M., 281, 221–225
9. Galbiati, F., Guzzi, F., Magee, A. I., Milligan, G., and Parenti, M. (1994) Biochem. J. 303, 679–686
10. Degtyarev, M. Y., Spiegel, A. M., and Jones, T. L. Z. (1994) J. Biol. Chem. 269, 30988–30993
11. Milligan, G., Parenti, M., and Magee, A. I. (1995) Trends Biochem. Sci. 20, 181–186
12. Wedegaertner, P. B., Chu, D. H., Wilson, P. T., Levis, M. J., and Bourne, H. R. (1995) J. Biol. Chem. 269, 25001–25008
13. Li, S., Okamoto, T., Chum, M., Sargiacomo, M., Casanova, J. E., Hansen, S. H., Nishimoto, I., and Lisanti, M. P. (1995) J. Biol. Chem. 270, 15693–15701
14. Edgerton, M. D., Chabert, C., Chollett, A., and Arkinstall, S. (1994) FEBS Lett. 354, 195–199
15. Richardson, A., Demolino-Mason, C., and Barnard, E. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10198–10202
16. Tian, W.-N., Duzic, E., Janvier, S. M., and Deth, R. C. (1994) Mol. Pharmacol. 45, 524–531
17. Georgoussi, Z., Milligan, G., and Zidoune, C. (1995) Biochem. J. 306, 71–75
18. Bertin, B., Friesmuth, M., Jockers, R., Strosberg, A. D., and Marullo, S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8827–8831
19. Wise, A., Carr, I. C., and Milligan, G. (1997) Biochem. J. 325, 17–21
20. Wise, A., Watson-Koken, M.-A., Rees, S., Lee, M., and Milligan, G. (1997) Biochem. J. 321, 721–728
21. Wise, A., Grassie, M. A., Parenti, M., Lee, M., Rees, S., and Milligan, G. (1997) Biochemistry, in press
22. Guyer, C. A., Horstman, D. A., Wilson, A. L., Clark, J. D., Cragoe, E. J., Jr., and Limbird, L. E. (1990) J. Biol. Chem. 265, 17307–17317
23. Gillard, N. P., Linton, C. J., Milligan, G., Carr, I. C., Patmore, L., and Brown, C. M. (1996) Br. J. Pharmacol. 117, 298P
24. Green, A., Johnson, J. L., and Milligan, G. (1990) J. Biol. Chem. 265, 5206–5210
25. McKenzie, F. R., and Milligan, G. (1990) Biochem. J. 267, 391–398
26. Kasiki, G., and Klee, W. A. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4185–4189
27. Kennedy, M. E., and Limbird, L. E. (1993) J. Biol. Chem. 268, 8003–8011
28. Wall, M. A., Coleman, D. E., Lee, E., Iniguez-Lluhi, J. A., Posner, B. A., Gilman, A. G., and Sprang, S. R. (1995) Cell 83, 1047–1058
29. Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E., and Sigler, P. B. (1996) Nature 379, 311–319
30. Milligan, G. (1988) Biochem. J. 255, 1–13
31. Senogles, S. E. (1994) J. Biol. Chem. 269, 23120–23127
32. Hunt, T. W., Carroll, R. C., and Peralta, E. G. (1994) J. Biol. Chem. 269, 25665–25670
33. Kisselev, O., Ermolaeva, M., and Gautam, N. (1995) J. Biol. Chem. 270, 25356–25358
34. Kisselev, O., Pronin, A., Ermolaeva, M., and Gautam, N. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9102–9106
35. Taylor, J. M., Jacob-Mosier, G. G., Lawton, R. G., VanDort, M., and Neubig, R. R. (1996) J. Biol. Chem. 271, 3336–3339
36. Taylor, J. M., Jacob-Mosier, G. G., Lawton, R. G., Remmers, A. E., and Neubig, R. R. (1994) J. Biol. Chem. 269, 27618–27624