Expression of a G Protein-coupled Receptor (GPCR) Leads to Attenuation of Signaling by Other GPCRs

EXPERIMENTAL EVIDENCE FOR A SPONTANEOUS GPCR CONSTITUTIVE INACTIVE FORM*

Received for publication, December 28, 2009, and in revised form, March 8, 2010 Published, JBC Papers in Press, March 18, 2010 DOI 10.1074/jbc.M109.099689

Maria Rosario Tubio‡, Natalia Fernandez‡§, Carlos Patricio Fitzsimons§, Sabrina Copsel‡§, Sergio Santiago‡, Carina Shayo¶, Carlos Davio‡¶, and Federico Monczor‡§

From the 4Laboratorio de Farmacología de Receptores, Cátedra de Química Medicinal, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 1113 Buenos Aires, Argentina, the 6Medical Pharmacology Department, Leiden/Amsterdam Center for Drug Research, Leiden University, 2300 Leiden, The Netherlands, the 11Laboratorio de Farmacología y Patología Molecular, Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina, and the 5Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

The idea of G protein-coupled receptors (GPCRs) coupling to G protein solely in their active form was abolished when it was found that certain ligands induce a G protein-coupled but inactive receptor form. This receptor form interferes with signaling of other receptors by sequestering G protein. However, the spontaneous existence of this receptor species has never been established. The aim of the present work was to evaluate the existence of the spontaneous conformation of the receptor inactive to G protein able to interfere with the response of other GPCRs. According to the law of mass action, receptor overexpression should lead to increased amounts of all spontaneously occurring species. Based on this, we generated Chinese hamster ovary (CHO-K1)-derived cell lines expressing various amounts of the human histamine H2 receptor. In these systems, the signaling of other endogenously and transiently expressed GPCRs was attenuated proportionally to human H2 receptor expression levels. G protein transfection specifically reverted this attenuation, strongly suggesting hijacking of the G protein from a common pool. Similar attenuation effects were observed when the β2-adrenergic receptor was overexpressed, suggesting that this is a more general phenomenon. Moreover, in human mammary MDA-MB-231 cells, a consistent increase in the constitutive H2 receptor activity could be detected in Chinese hamster ovary (CHO-K1) cells (7).

The histamine H2 receptor (H2R) is an extensively characterized member of the GPCR family, which in most systems couples to Gi proteins to activate adenylyl cyclase (3–6). Compared with other GPCRs, the H2R is unique in that the wild-type receptor possesses a remarkably high degree of constitutive activity. With a receptor density of 300 fmol/mg protein, constitutive H2 receptor activity could be detected in Chinese hamster ovary (CHO-K1) cells (7).

The notion that GPCRs also signal without an external chemical trigger, i.e. in a constitutive or spontaneous manner, resulted in a paradigm shift in the field of GPCR pharmacology. Before the discovery of constitutive GPCR activity, efficacy was considered only as a positive property (i.e. producing an increased receptor activity, and only ligand-induced activation of receptors was thought to induce G protein activity), but with the discovery of spontaneous activation of G proteins by unliganded receptors came the prospect of ligands that selectively inhibit this spontaneous activation, specifically denominated inverse agonists.

In an attempt to understand GPCR activation mechanisms, several receptor occupancy models have been developed (8). The first that explicitly considered constitutive activity was the extended ternary complex (ETC) model presented by Samama et al. (9), which includes two distinct conformational states of the receptor, an active (R*) and an inactive (R) state, that exist in equilibrium even in the absence of drugs. This spontaneous equilibrium determines the level of constitutive activity because in the ETC model, only R* is able to couple to the G protein and is considered the responsible of basal activity (R*G).

G protein-coupled receptors (GPCRs)2 form a large and functionally diverse superfamilly of proteins that transduce signals across cell membranes. Although much is known about structural features of GPCRs involved in ligand recognition and G protein binding, the actual mechanism underlying GPCR signaling remains unclear.

Traditionally, agonist occupancy of GPCRs is believed to result in a conformational change in the receptor, leading to activation of G proteins (1). However, in genetically engineered systems where receptors can be expressed at high densities, Costa and Herz (2) noted that high levels of receptor expression uncovered the existence of a population of spontaneously (unliganded) active receptors, resulting in an elevated basal response in the system.

The histamine H2 receptor (H2R) is an extensively characterized member of the GPCR family, which in most systems couples to Gi proteins to activate adenylyl cyclase (3–6). Compared with other GPCRs, the H2R is unique in that the wild-type receptor possesses a remarkably high degree of constitutive activity. With a receptor density of 300 fmol/mg protein, constitutive H2 receptor activity could be detected in Chinese hamster ovary (CHO-K1) cells (7).

The notion that GPCRs also signal without an external chemical trigger, i.e. in a constitutive or spontaneous manner, resulted in a paradigm shift in the field of GPCR pharmacology. Before the discovery of constitutive GPCR activity, efficacy was considered only as a positive property (i.e. producing an increased receptor activity, and only ligand-induced activation of receptors was thought to induce G protein activity), but with the discovery of spontaneous activation of G proteins by unliganded receptors came the prospect of ligands that selectively inhibit this spontaneous activation, specifically denominated inverse agonists.

In an attempt to understand GPCR activation mechanisms, several receptor occupancy models have been developed (8). The first that explicitly considered constitutive activity was the extended ternary complex (ETC) model presented by Samama et al. (9), which includes two distinct conformational states of the receptor, an active (R*) and an inactive (R) state, that exist in equilibrium even in the absence of drugs. This spontaneous equilibrium determines the level of constitutive activity because in the ETC model, only R* is able to couple to the G protein and is considered the responsible of basal activity (R*G).
A further modification of the ETC model is the cubic ternary complex model (CTC) (10–12), that extends the ETC model by allowing G proteins to interact with receptors in both their active and inactive states (i.e., R*G and R格). Although the development of the ETC model was made necessary by experimental observations, the CTC model was originally proposed in an attempt to explore theoretically the mathematical and pharmacological implications that can be derived from permitting G proteins to interact with receptors in their inactive and active forms. Thus, the CTC model was the culmination of a trend in increasing model complexity and statistical and thermodynamic completeness.

However, there is a growing body of evidence suggesting that the CTC model is the only one capable of explaining some experimental observations concerning the mechanism of action of certain inverse agonists. Inverse agonists may act by binding to an inactive, G protein-coupled form of the receptor, decreasing basal activity of the specific GPCR of interest but also in some cases the activity of other GPCRs that signal through the same G protein, via a proposed “molecular kidnapping mechanism” (13–15).

According to the law of mass action, receptor overexpression leads to an increased amount of all spontaneously occurring species. Hence, receptor overexpression should uncover a receptor species spontaneously coupled to G protein but inactive, able to interfere with other GPCRs that signal through the same G protein pool.

In this study, aiming to characterize inactive spontaneously GPCR species experimentally, we generated five CHO-K1 cell clones stably transfected with the human histamine H2R. These clones express different and increasing amounts of the receptor and respond to ligand stimulation with an unaltered pharmacological profile.

Surprisingly, in these clones the signaling of other Gα-coupled receptors is attenuated proportionally to the H2R expression levels. Similar results were obtained when another Gα-coupled receptor, B2-adrenergic receptor (βAR), was overexpressed, indicating that this phenomenon is not restricted to histamine receptors. Moreover, G protein transfection specifically reverted this interference, strongly suggesting that the mechanism is related to G protein hijacking. Finally, we observed that knocking down the expression of endogenously expressed βAR leads to an increased ligand-induced response of other Gα-coupled receptors, indicating that this phenomenon is not restricted only to overexpression systems.

These results indicate that the CTC model prediction, stating that GPCRs spontaneously exist not only as a constitutive active form (R*G) but also as a constitutive inactive form (RG), was verified experimentally by its ability to sequester G protein and interfere with the signaling of other GPCRs. This phenomenon could have serious physiological implications because it was observed not only in genetically manipulated systems, but also with endogenously expressed receptors.

**EXPERIMENTAL PROCEDURES**

**Materials**—CHO-K1 dhfr and MDA-MB-231 cells were obtained from the American Type Culture Collection. Cell culture medium, antibiotics, isobutylmethylxanthine (IBMX), cAMP, HT medium supplement, G418, and bovine serum albumin were obtained from Sigma. Amthamine, isoproterenol, prostaglandin E2 (PGE2), salmon calcitonin (sCT), and tiotidine were from Tocris Bioscience (Ellisville, MO). \([3H]cAMP\) (31 Ci/mmol), \([3H]\)tiotidine (75 Ci/mmol), and \([3H]\)CGP12177 (30 Ci/mmol) were purchased from PerkinElmer Life Sciences. Three siRNAs for βAR were purchased from Invitrogen (ADRB2 Stealth Select RNAiTM, HSS100258, HSS100259, and HSS100260). Other chemicals used were of analytical grade. pcDNA3-βAR was a generous gift from Dr. M. Levin (INGEBI, CONICET, Argentina). pcDNA3Gαs plasmids were generous gifts from Dr. O. Cosso (Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina). Human H2R was previously cloned into the eukaryotic expression vector pCEFL (16).

**Cell Culture and Transfection**—All cells were grown at 37 °C in a humidified 5% CO2 incubator. CHO-H2R and CHO-mock cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 2mM L-glutamine, 100 mM hypoxanthine, 16 μM thymidine, 50 g/ml Gentamicin, and 0.8 mg/ml G418. Parental CHO-K1 cells were cultured in the same medium without G418. MDA-MB-231 cells were grown in Dulbecco’s modified Eagle’s medium-F12 medium containing 10% fetal calf serum and 50/μl Gentamicin.

For transfection CHO-K1 cells were grown to 80–90% confluence. cDNA constructs were transfected into cells using Lipofectamine 2000. The transfection protocol was optimized as recommended by the supplier (Invitrogen). After transfection, five stable clones with different H2R levels were established by G418 selection. A separate single clone containing the empty vector was selected under the same conditions (CHO-mock).

Transfections with double stranded siRNA targeting βAR at 20 nm concentration were also performed using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instructions. The three different sequences provided were used separated or pooled. As control, nontargeting scrambled siRNA was used. Transiently cDNA or siRNA-transfected cells were assayed 48 h after transfection. Rceptor expression was evaluated by specific radioligand binding assay as described below.

**cAMP Assays**—Concentration-response assays were performed by incubating the cells for 3 min in culture medium supplemented with 1 mM IBMX at 37 °C, followed by a 7-min exposure to different concentrations of ligands. The reaction was stopped by the addition of ethanol. The ethanolic phase was then dried and the residue resuspended in 50 μM Tris-HCl, pH 7.4, 0.1% bovine serum albumin. cAMP content was determined by competition of \([3H]\)cAMP for protein kinase A, as described previously (17).

**Radioligand Binding Assay**—Triplicate assays were performed in 50 mM Tris-HCl, pH 7.4. For saturation studies, 104 CHO-mock, CHO-H2R, or MDA-MB-231 cells/well of a 48-well cluster plate were incubated for 40 min at 4 °C with increasing concentrations of \([3H]\)tiotidine, ranging from 0.4 to 240 nM in the absence or in the presence of 1 μM unlabeled tiotidine or for 4 h at 4 °C with increasing concentrations of \([3H]\)CGP12177, ranging from 20 to 0.02 nM in the absence or in the presence of 100 nM isoproterenol. The incubation was...
stopped by dilution with 3 ml of ice-cold 50 mM Tris-HCl, pH 7.4. After three washes with 3 ml of ice-cold buffer, the bound fraction was collected in 200 μl of ethanol. Experiments with intact cells were performed at 4 °C to avoid ligand internalization. The kinetic studies performed with 2 nM [3H]tiotidine at 4 °C showed that the equilibrium was reached at 30 min and sustained for 4 h (data not shown).

Statistical Analysis—Binding data and sigmoidal dose-response fittings were performed with GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, CA). One-way analysis of variance followed by the Dunnett’s post test was performed using GraphPad InStat version 3.01. Specific binding was calculated by subtraction of nonspecific binding from total binding.

RESULTS

H2R Overexpression Interferes with the Signaling of Other Endogenously Expressed GPCRs—H2R constitutive activity (7) and the triggering of cellular mechanisms tending to compensate the activity of the signaling pathways when it is overexpressed (18) have been described. With the aim of characterizing the effect of H2R overexpression on signaling of other Gs-coupled receptors, we established CHO-K1 cells clones stably transfected with cDNA encoding H2R. Several clones were isolated, and five were selected based on their H2R amounts (C1, C2A, C2B, C3, and C4). [3H]Tiotidine binding assays performed on these clones yielded different Bmax values ranging from about 1.3 × 10^5 to 2 × 10^6 sites/cell (Table 1). We observed for C1, C2A, and C2B the two different binding sites previously described for H2 (14, 19, 20): the high affinity site corresponding to the G protein-coupled forms of the H2R (about 20% of total sites number) and the low affinity site corresponding to the G protein-uncoupled states. However, in C3 and C4 clones that expressed the highest amounts of receptors, we observed only the low affinity binding site (Table 1). The lack of the high affinity site in these clones can be interpreted assuming that G protein sequestering by GPCRs

| Clone | Bmax H (10^3 sites/cell) | Kd H | Bmax L (10^3 sites/cell) | Kd L | H/L affinity ratio |
|-------|-------------------------|------|-------------------------|------|-------------------|
| C1 (4) | 22.9 ± 1.5 | 5.28 ± 0.47 | 109.6 ± 9.9 | 23.54 ± 1.98 | 17.33 |
| C2A (4) | 61.9 ± 5.9 | 2.04 ± 0.31 | 205.9 ± 18.9 | 17.85 ± 2.12 | 21.13 |
| C2B (4) | 50.9 ± 5.5 | 1.74 ± 0.21 | 237.6 ± 19.0 | 21.64 ± 2.41 | 17.60 |
| C3 (3) | 125.4 ± 49 | 30.76 ± 2.98 | 2063 ± 70 | 26.15 ± 2.56 | 9.99 |
| C4 (3) | 2063 ± 70 | 26.15 ± 2.56 | 2063 ± 70 | 26.15 ± 2.56 | 9.99 |

G Protein Sequestering by GPCRs

FIGURE 1. Dose-dependent cAMP production by amphetamine treatment in H2-transfected CHO cells. A, control cells (mock transfected, □) and clones C1 (■), C2A (▲), C2B (△), C3 (●), and C4 (●); cells were incubated for 7 min with increasing concentrations of amphetamine at 37 °C in the presence of 1 mM IBMX; cAMP levels were determined. Data are the mean ± S.D. (error bars) of triplicate assays and representative of at least six independent experiments.

B, variation of fitted parameters for cAMP dose-response curves (maximal responses (■), basal levels (▲), and pEC50) with H2R number is shown. Data are the mean ± S.E. of six independent experiments and are best fit by a hyperbola (maximal responses) or a straight line (basal levels and pEC50) with slope significantly different from zero (p < 0.01). Dotted lines represent the 95% confidence interval of the curve.

The table shows the mean ± S.E.; the number of determinations (n) is in parentheses.
proteins are in limiting quantity with respect to the amounts of receptor overexpressed. Concentration-response curves performed with the specific H2 agonist amphetamine showed an increase in cAMP basal levels and a decrease in pEC50 values according to the increment on receptor amount. This behavior agrees with predictions made, using simpler operational models (21) (Fig. 1).

As stated in the Introduction, if a receptor is overexpressed, all spontaneous species should be incremented as well, according to its probability of occurrence. Considering this, receptor overexpression may also lead to an increase in the hypothetical species corresponding to an unliganded inactive G protein-coupled form of the receptor.

According to the results obtained for [3H]thiotidine binding, G protein amounts are in a limiting number regarding H2 receptors. As a consequence, overexpression of a particular receptor may cause a G protein kidnapping and an interference in the response of other GPCRs that signal through the same subfamily of G proteins.

Hence, to test whether H2R expression affects the signaling of other GPCRs that transduce their signals through the same G protein, we evaluated the ability of signaling of CHO-K1 endogenously expressed Gaα5-coupled receptors. To do this, we confirmed the presence and the functionality of CT and PGE2 receptors that were previously described on the CHO-K1 cell line (22, 23) (Fig. 2).

The only presence of the H2R is able to reduce, in a receptor number-dependent manner, the CTR and PGE2R signaling. This interference consists in a reduction of the ligand-induced maximal responses without significantly affecting the pEC50 (Fig. 2 insets). This can be predicted with any model of receptor occupancy considering a limiting and diminishing G protein amounts available for signaling. As shown in Fig. 2 insets, the decrease in maximal responses is best fit to an exponential decay equation, and the interference is more intense for the PGE2 system, indicating that the propensity to be interfered is different for each GPCR. It is worth noting that, in saturation binding assays, the number and the affinity constants of the aforementioned receptors remained unchanged (data not shown).

Overall, these results may be explained by the kidnapping of available G protein in an inactive form by overexpressed H2R.

To confirm this hypothesis, we attempt to overexpress G protein to increase its availability.

Ga Overexpression Reverses the H2R Interference on Calcitonin and PGE2 Signaling—The results described above may be explained by the kidnapping of available G protein in an inactive form by overexpressed H2R. If this hypothesis was right, an increase in the amounts of G proteins of this family would counteract the effect of H2R on the sCT and PGE2 response in CHO clones.

Fig. 3 shows that the interference was abolished on C1, C2A, and C2B clones, both for CTR and PGE2R ligand-induced signaling but that this recovery effect lost efficacy on the clones where H2R number is higher (C3 and C4 clones). These results...
confirm that the interference observed could be due to the kidnapping of the G protein by H2R and strengthen the concept that the stoichiometry of the different signaling partners is crucial to determine the signaling ability of a system.

**H2R Expression Also Attenuates the Signaling of Other Exogenously Expressed GPCRs**—To evaluate further the attenuating effect of H2R expression, considering a role of an endogenous regulation as partially responsible for the observed interference, we also studied the signaling of a heterologously expressed GPCR, the βAR. When βAR was transiently transfected into the different CHO-H2R clones we observed no differences on receptor expression by [3H]CGP12177 saturation binding experiments (data not shown). However, we could observe that its signaling is also attenuated in an H2R number-dependent manner. When plotted as isoproterenol maximal response versus H2R number, the data also best fit a one-phase exponential decay, but the curve was shifted to the right, indicating that H2R is less efficacious in interfering with the βAR signaling (Fig. 4). When βAR is expressed on CHO cells, its presence is also able to attenuate sCT and PGE2 signaling (Fig. 5, A and B), and when expressed on CHO-H2R cells it is able to interfere with H2R-mediated response (Fig. 5C), indicating that this interference phenomenon is not restricted to any chosen receptor pair.

As shown before, H2R overexpression led to a concomitant increase on second messenger basal levels (Fig. 1B). However, surprisingly, the same effect was not observed when βAR is overexpressed. In the latter case, the basal levels of all clones were unchanged or diminished (Fig. 4A). To evaluate this striking effect better, we overexpressed Goα protein. Under these experimental circumstances, we were able to observe the expected increase on cAMP levels, but βAR co-expression was capable of diminishing this magnified basal response (Fig. 6). This was tested using three different Goα and βAR plasmid concentrations, and the results were reproducible for every condition (data not shown). These results may be indicative of the natural tendency of a GPCR to adopt distinct spontaneous conformations, showing that βAR has more tendency than H2R to adopt a spontaneous conformation able to bind G proteins in an inactive state.

**Knockdown of Endogenous βAR Augments the Response of Other GPCRs**—Previously, it has been reported that although heterologously transfected GPCRs share a common G protein pool, endogenously expressed receptors by naïve cells activate different pools of G protein (24). Therefore, to evaluate whether this interference phenomenon is restricted to exogenously expressed receptors, we utilized a cell line that endogenously expresses the set of Goα-coupled GPCRs examined in this work. We chose MDA-MB-231 cells, a human mammary carcinoma cell line that endogenously expresses βAR and H2R (25, 26). In this cell line, transfection with siRNA targeted against βAR diminished membrane receptor number approximately 80% when measured by saturation binding experiments and decreased isoproterenol-induced cAMP levels 60%. However, although siRNA transfection did not change the H2R number, the H2R response was significantly increased (22.01 ± 2.73 versus 51.82 ± 3.47 pmol/well), consistent with our hypothesis (Fig. 7). Furthermore, the potentiating effect of the βAR-specific siRNA was observed as well for other endogenously expressed receptors such as CTR and PGE2R (27, 28) (Fig. 7). These results support the fact that the only presence of a GPCR can affect the response of another receptor not only in genetically manipulated cells, but also in endogenous expression systems.

**DISCUSSION**

Three main conclusions can be drawn from our studies. First, human H2R overexpression in CHO-K1 cells shows that H2R is able to interfere with CTR and PGE2R signaling. Second, transient expression of βAR shows that this is not exclusive of H2R. Third, experiments performed knocking down the endogenous expression of βAR in MDA-MB-231 cells show that this phenomenon is not restricted to overexpression systems and that it can be evidenced in physiological conditions.

We have previously described that certain inverse agonists acting on histaminergic receptors interfere with the signaling of other receptors that share common Goα subunits (14, 15). This interference is thought to be caused by the ligand-induced stabilization of a G protein-coupled form of the receptor, which is
Assuming the collision-coupling model of membrane receptor signaling (32, 33), which allows for receptors and G proteins to diffuse freely in the membrane, if the latter are in a limiting number, it is possible to anticipate an interference of a GPCR with the signaling of other receptors based on the redistribution of the subabundant G proteins. That may be the case for the reported ligand competitive behavior, in which the stimulation achieved by the addition of two agonists acting on differentGPCRs is less than the sum of the activation caused by the individual receptors alone (e.g. CB1 and μ opioid receptors) (24). It is worth noting that in that work, the authors concluded that exogenously transfected receptors share a common G protein pool, whereas endogenously expressed receptors interact with distinct pools. Contrarily, the results obtained knocking down the expression of endogenous βAR indicate that the set of GPCRs studied share a common G protein pool, on which a receptor could signal at the expense of the others. In accordance with our results, it has been found that ligand-activated V1 vasopressin receptor and α₁-adrenoreceptor endogenously expressed on rat hepatocytes and receptors for the chemotactic factors fMet-Leu-Phe and C5a endogenously expressed on human HL 60 cells compete for the same limited pool of G proteins (34, 35).

Remarkably, our experiments show that solely the expression of a GPCR dampens the agonist-induced signaling of endogenously or heterologously expressed receptors. Moreover, overexpression of the βAR is able to diminish elevated cAMP levels resulting from Gα₅ overexpression, strengthening our proposal of the G protein-coupled but inactive receptor form.

In line with our results, it has been described that 5HT7 serotonin receptor attenuates adenylyl cyclase activation by βAR and prostanoid EP receptor. However, in that case, neither Gα₅ nor adenylyl cyclase overexpression is able to reverse the interference effect, indicating that the mechanism underlying their observations is different (36). Furthermore, Stephan and co-workers have shown that the constitutive abnormal signaling of mutated yeast pheromone receptors Ste2p and Ste3p is suppressed upon co-expression with wild-type but not G protein coupling-defective receptors, suggesting that wild-type receptors may sequester a limiting pool of G proteins (37). Considering the results obtained on the carcinoma cell line, the phenomenon herewith described could have serious implications regarding the effects of an unbalance of protein expression on receptor signaling.
Hasseldine and co-workers (38) have described the signaling of the \( \beta H2R \)-adrenergic system in TG4 mice, a strain that specifically overexpresses \( \beta H2R \) in cardiac tissue. In this system, as a result of its overexpression, \( \beta H2R \) couples simultaneously to \( Gs \) and \( Gi \) pathways, but for yet unknown reasons \( \beta H1R \) cardiac signaling is dampened in the transgenic mice. This surprising result could be interpreted in terms of the G protein-hijacking mechanism proposed, bringing out the possible physiological relevance.

As mentioned above, GPCRs are overexpressed in various malignancies. For instance, there has been conducted an in silico approach demonstrating overexpression of several GPCRs in primary tumor cells, including chemokine receptors and protease-activated receptors, neuropeptide receptors, adenosine A2B receptor, P2Y purinoceptor, calcium-sensing receptor, and metabotropic glutamate receptors. Analysis of cancer samples in different disease stages also suggests that some GPCRs, such as endothelin receptor A, may be involved in early tumor progression, and others, such as CXCR4, may play a critical role in tumor invasion and metastasis (39).

Besides cancer, there have been described other conditions in which receptors are overexpressed (e.g. schizophrenia and dopamine D4 receptors) (40) and presumably H2R and certain heart disease states (41)). In those cases, as well as some easily anticipated consequences (i.e. an elevation on second messen-
In summary, we have shown that the human H2R and βAR have the ability to block the signaling by other endogenous or exogenously expressed Gs-coupled receptors. Sequestration of G proteins by these receptors is well accommodated by CTC model, and our study suggests that GPCRs may act as proteins controlling the signaling of other receptors sharing a common and limiting G protein pool.

REFERENCES

1. Perez, D. M., and Karnik, S. S. (2005) Pharmacol. Rev. 57, 147–161
2. Costa, T., and Herz, A. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 7321–7325
3. Gantz, I., Munzert, G., Tashiro, T., Schäffer, M., Wang, L., Delvalle, J., and Yamada, T. (1991) Biochem. Biophys. Res. Commun. 178, 1386–1392
4. Gantz, I., Schäffer, M., Delvalle, J., Logsdon, C., Campbell, V., Uhler, M., and Yamada, T. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 429–433
5. Ruat, M., Traiffort, E., Arrang, J. M., Leurs, R., and Schwartz, J. C. (1991) Biochem. Biophys. Res. Commun. 179, 1470–1478
6. Traiffort, E., Vizuete, M. L., Tardivel-Lacombe, J., Soulé, E., Schwartz, J. C., and Ruat, M. (1995) Biochem. Biophys. Res. Commun. 211, 570–577
G Protein Sequestering by GPCRs

7. Smit, M. J., Leurs, R., Alewijnse, A. E., Blauw, J., Van Nieuw Amerongen, G. P., Van De Vrede, Y., Roovers, E., and Timmerman, H. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 6802–6807
8. Kenakin, T. (2004) Trends Pharmacol. Sci. 25, 186–192
9. Samama, P., Cotecchia, S., Costa, T., and Lefkowitz, R. J. (1993) J. Biol. Chem. 268, 4625–4636
10. Weiss, J. M., Morgan, P. H., Lutz, M. W., and Kenakin, T. P. (1996) J. Theor. Biol. 178, 151–167
11. Weiss, J. M., Morgan, P. H., Lutz, M. W., and Kenakin, T. P. (1996) J. Theor. Biol. 178, 169–182
12. Weiss, J. M., Morgan, P. H., Lutz, M. W., and Kenakin, T. P. (1996) J. Theor. Biol. 181, 381–397
13. Bouaboula, M., Perrachon, S., Milligan, L., Canat, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Peccue, F., Lupker, J., Maffrand, J. P., Le Fur, G., and Casellas, P. (1997) J. Biol. Chem. 272, 22330–22339
14. Monczor, F., Fernandez, N., Legnazzi, B. L., Riveiro, M. E., Baldi, A., Shayo, C., and Davio, C. (2003) Mol. Pharmacol. 64, 512–520
15. Fitzsimons, C. P., Monczor, F., Fernandez, N., Shayo, C., and Davio, C. (2004) J. Biol. Chem. 279, 34431–34439
16. Shayo, C., Fernandez, N., Legnazzi, B. L., Monczor, F., Mladovan, A., Baldi, A., and Davio, C. (2001) Mol. Pharmacol. 60, 1049–1056
17. Davio, C. A., Cricco, G. P., Bergoc, R. M., and Rivera, E. S. (1995) Biochem. Pharmacol. 50, 91–96
18. Monczor, F., Fernandez, N., Riveiro, E., Mladovan, A., Baldi, A., Shayo, C., and Davio, C. (2006) Biochem. Pharmacol. 71, 1219–1228
19. Rising, T., and Norris, D. (1985) in Frontiers in Histamine Research (Ganellin, C., and Schwartz, J., eds) pp. 61–68, Pergamon Press, London
20. Batzri, S., and Harmon, J. W. (1986) Pharmacol. Rev. 32, 241–247
21. Black, J. W., and Leff, P. (1983) Proc. R. Soc. Lond. B Biol. Sci. 220, 141–162
22. George, S. E., Bungay, P. J., and Naylor, L. H. (1997) J. Neurochem. 69, 1278–1285
23. Horie, K., and Insel, P. A. (2000) J. Biol. Chem. 275, 29433–29440
24. Shapira, M., Vogel, Z., and Sarne, Y. (2000) Cell. Mol. Neurobiol. 20, 291–304
25. Slotkin, T. A., Zhang, J., Dancel, R., Garcia, S. J., Willis, C., and Seidler, F. J. (2000) Breast Cancer Res. Treat. 60, 153–166
26. Medina, V., Cricco, G., Nuñez, M., Martin, G., Mohamad, N., Correa-Fiz, F., Sanchez-Jimenez, F., Bergoc, R., and Rivera, E. S. (2006) Cancer Biol. Ther. 5, 1462–1471
27. Nakamura, M., Han, B., Nishishita, T., Bai, Y., and Kakudo, K. (2007) J. Mol. Endocrinol. 39, 375–384
28. Timoshenko, A. V., Xu, G., Chakrabarti, S., Lala, P. K., and Chakraborty, C. (2003) Exp. Cell Res. 289, 265–274
29. Piomelli, D., Pilon, C., Giros, B., Sokoloff, P., Martres, M. P., and Schwartz, J. C. (1991) Nature 353, 164–167
30. Jordan, B. A., and Devi, L. A. (1999) Nature 399, 697–700
31. Cilluffo, M. C., Xia, S. L., Farahbakhsh, N. A., and Fain, G. L. (1998) Invest. Ophthalmol. Vis. Sci. 39, 1429–1435
32. Stickle, D., and Barber, R. (1996) Biochim. Biophys. Acta 1310, 242–250
33. Shea, L., and Linderman, J. J. (1997) Biochem. Pharmacol. 53, 519–530
34. Dasso, L. L., and Taylor, C. W. (1992) Mol. Pharmacol. 42, 453–457
35. Wieland, T., Gierschik, P., and Jakobs, K. H. (1992) Naunyn. Schmiedebergs. Arch. Pharmacol. 346, 475–481
36. Andressen, K. W., Norum, J. H., Levy, F. O., and Krobert, K. A. (2006) Mol. Pharmacol. 69, 207–215
37. Stefan, C. J., Overton, M. C., and Blumer, K. J. (1998) Mol. Biol. Cell 9, 885–899
38. Hasseldeine, A. R. G., Harper, E. A., and Black, J. W. (2003) Br. J. Pharmacol. 138, 1358–1366
39. Li, S., Huang, S., and Peng, S. B. (2005) Int. J. Oncol. 27, 1329–1339
40. Seeman, P., Guan, H. C., and Van Tol, H. H. (1993) Nature 365, 441–445
41. Matsuda, N., Jesmin, S., Takahashi, Y., Hatta, E., Kobayashi, M., Matsuyama, K., Kawakami, N., Sakuma, I., Gando, S., Fukui, H., Hattori, Y., and Levi, R. (2004) J. Pharmacol. Exp. Ther. 309, 786–795