G Protein-coupled Receptor Kinase Regulates Dopamine D3 Receptor Signaling by Modulating the Stability of a Receptor-Filamin-β-Arrestin Complex

A CASE OF AUTORECEPTOR REGULATION*

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In addition to its postsynaptic role, the dopamine D3 receptor (D3R) serves as a presynaptic autoreceptor, where it provides continuous feedback regulation of dopamine release at nerve terminals for processes as diverse as emotional tone and locomotion. D3R signaling ability is supported by an association with filamin (actin-binding protein 280), which localizes the receptor with G proteins in plasma membrane lipid rafts but is not appreciably antagonized in a classical sense by the ligand-mediated activation of G protein-coupled receptor kinases (GRKs) and β-arrestins. In this study, we investigate GRK-mediated regulation of D3R-filamin complex stability and its effect on D3R-G protein signaling potential. Studies in HEK-293 cells show that in the absence of agonist the D3R immunoprecipitates in a complex containing both filamin A and β-arrestin2. Moreover, the filamin directly interacts with β-arrestin2 as assessed by immunoprecipitation and yeast two-hybrid studies. With reductions in basal GRK2/3 activity, an increase in the basal association of filamin A and β-arrestin2 with D3R is observed. Conversely, increases in the basal GRK2/3 activity result in a reduction in the interaction between the D3R and filamin but a relative increase in the agonist-mediated interaction between β-arrestin2 and the D3R. Our data suggest that the D3R, filamin A, and β-arrestin form a signaling complex that is destabilized by agonist- or expression-mediated increases in GRK2/3 activity. These findings provide a novel GRK-based mechanism for regulating D3R signaling potential and provide insight for interpreting D3R autoreceptor behavior.

The D3 dopamine receptor (D3R), in addition to serving in a classical postsynaptic sensory role, is also a presynaptic autoreceptor that is predominantly expressed in parts of the brain controlling behavior, including that involved with emotion and movement (1). The D3R is homologous to four other dopamine receptor subtypes (2) but is closest in expression and function to the D2R (1, 3). The D2 and D3 autoreceptors have overlapping distributions in the brain (2, 4, 5), where they regulate presynaptic release of dopamine (DA) by at least three different mechanisms: by affecting rates of intracellular DA synthesis, by mediating vesicular secretion of DA at the plasma membrane, and by modulating reuptake of dopamine at plasma membrane DA transporters (1, 6). D3R has a higher affinity for DA than D3Rs, and experiments with D3R knock-out mice suggest that in the striatum/nucleus acumbens the D3R may regulate DA release at lower DA concentrations than D2R by modulating DA secretion rates rather than DA synthesis or DA transporter activity (1).

The G protein-mediated signaling of G protein-coupled receptors (GPCR) in general and D2 and D3Rs in particular should terminate from arrestin protein binding to receptor intracellular loop or C-tail residues that are GPCR kinase (GRK)-phosphorylated (7–9). The subsequent trafficking of arrestin-receptor complexes to clathrin-rich regions of plasma membrane and clathrin-coated pits (CCPs) depends on the extent of GRK phosphorylation (10) and results in a sustained blockade of G protein coupling, the establishment of arrestin-dependent non-G protein-mediated signaling, and the endocytosis of arrestin-receptor complexes (7, 8, 11). CCP-initiated recycling of dephosphorylated receptors contributes to relief of the blockade in G protein signaling and a reinitiation of membrane signaling competence (12, 13). Autoreceptors that function as permanent sensors of extracellular neurotransmitter concentrations may require alternative mechanisms to regulate the transition between the classical G protein signaling and desensitized states observed for receptors with more conventional signaling responsibilities. In fact, although D3Rs can be induced to internalize to a small degree, the large majority of D3Rs remain at the plasma membrane and fail to internalize appreciably in CCPs after brief periods of DA stimulation (3).

The D3R and D3R undergo remarkably different degrees of arrestin-directed trafficking to CCPs (3). The molecular determinants including GRK phosphorylation sites underlying CCP association are confined to their relatively large third intracellular loops as demonstrated by experiments in which the loops were interchanged between receptors (3). In contrast to this post signaling behavior, the D3R and D3R share a requirement for β2AR, β3-adrenergic receptor; HA, hemagglutinin; GFP, green fluorescent protein.
GRK Regulates a D₃R, Filamin, β-Arrestin Complex

EXPERIMENTAL PROCEDURES

Materials—Human embryonic kidney cells (HEK-293) and COS-7 cells were obtained from the American Type Culture Collection (Manassas, VA). Cell culture reagents were from Cellgro (Herndon, VA) or Invitrogen. (Palo Alto, CA). Flag-M2 and HA epitopes (3) were purchased from Clontech (Palo Alto, CA), and library screening was carried out as previously described (28).

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in response to agonist stimulation of GRK activity as opposed to the 50% agonist-mediated increases in phosphorylation apparent for the D3R (Fig. 1B, right panel), which was used as a positive control.

**Dopamine D3 Receptor Is Associated Basally with Arrestin—**
GRK-mediated receptor phosphorylation changes GPCR affinity for arrestins. Since the phosphorylation status of the D3R in the absence of agonist was unexpectedly high compared with that of βAR or D2R, the interaction between the D3R and β-arrestin was assessed for the basal state. A Western blot for β-arrestins isolated from D3R immunoprecipitates of COS-7 cells shows that the D3R associates with β-arrestin2 under basal conditions (Fig. 1C, lane 2). Agonist exposure does not significantly change the degree of this association (Fig. 1C, lanes 2 and 3). A negligible agonist-induced D3R phosphorylation is consistent with both the observed lack of change in the co-immunoprecipitation experiments (Fig. 1C, lane 3), and the absence of translocation of β-arrestins to D3R at 5 min after agonist treatment (Fig. 1D, upper panel).

**Exogenous GRK Expression Reduces the Basal Localization of the D3R with β-Arrestin while Facilitating Agonist-induced β-Arrestin Recruitment—**We have observed both in COS-7 and HEK-293 cells that DA treatment does not augment the basal association of D3R with β-arrestin. However, an enhancement of GRK activity in these cells resulting from transfection of a plasmid encoding GRK noticeably increased the relative degree of agonist-dependent association of β-arrestins with D3R in clathrin-coated pits as observed by microscopy (Fig. 1D, lower panel). A corresponding immunoprecipitation experiment to determine the association of β-arrestin with the D3R in the

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**FIG. 1. Characterization of the phosphorylation and β-arrestin binding of the dopamine D3 receptor.** A, representative image of the photoaffinity-labeled long form of the D3R and D2R that were analyzed by SDS-PAGE. The receptor proteins were labeled with 500 pm [125I]NAPS in the presence or absence of a 1 μM concentration of the competitive dopamine receptor ligand (+)-butaclamol (28). Molecular mass markers representing kDa (kDa) are to the right of the gel. B, SDS-PAGE of phosphorylated HA-βAR and HA-D3Rs that were exposed to 10 μM isoproterenol (ISO) or DA for 5 min. Receptors were immunoprecipitated from HEK-293 cells transfected with 2 μg of receptor cDNAs in pCMV5. Receptor expression was determined by radioligand binding, and the same amount of receptor protein was loaded in each gel lane. Phosphorylated D3R bands were not observed in mock-transfected HEK-293 cells (data not shown). C, association between FLAG-tagged D3R and β-arrestin2 in COS-7 cells transfected with 3 μg of receptor cDNA in pCMV5 and with 2 μg of β-arrestin2 cDNA in pCMV5. Mock group was transfected with 4 μg of empty vector, pCMV5. Cells were treated with 1 μM dopamine for 15 min and lysed in radioimmune precipitation buffer. Immunoprecipitates obtained in radioimmune precipitation buffer were analyzed by SDS-PAGE with immunoblotting performed using antibodies to β-arrestin at a 1:4,000 dilution or using M2 FLAG antibodies at a 1:1,000 dilution. D, confocal microscopy images that demonstrate the extent of dopamine-mediated β-arrestin2-GFP translocation to the D3R in the absence and presence of GRK3. HEK-293 cells were co-transfected with the D3R (expressing ~5 pmol/mg protein) and with β-arrestin2-GFP, or with or without 2 μg of GRK3-pRK5. Cells in 2 ml of minimal essential medium containing 20 mM HEPES (pH 7.4) were stimulated with 10 μM dopamine for 5 min, and images were then obtained using a Zeiss laser-scanning confocal microscope (LSM-510).
presence of transfected GRK3 and DA treatment is shown in Fig. 2A (lanes 2 and 3). The results are consistent with the microscopic observations showing that arrestin translocation is observable in coated pits under these conditions (Fig. 1D, lower panels).

The co-expression of GRKs appears to decrease the basal association of β-arrestins with D₃Rs. This is evident from the Western blots showing the amounts of β-arrestin1 and -2 that were immunoprecipitated with the FLAG-tagged D₃R in the absence of agonist (Fig. 2B, lane 2 versus lane 3 and lane 4 versus lane 5). We observed above that agonist can drive D₃Rs to CCPs in the presence of elevated GRKs. To show that a similar behavior occurs under basal conditions, we trapped D₃Rs in CCPs using the endocytosis inhibitor dynamin1-K44A.

As shown in Fig. 2C, dynamin1-K44A alone did not have any effect on the subcellular localization of β-arrestin2-GFP. However, co-expression of GRK2 or GRK3 caused the accumulation of β-arrestin2-GFP at the plasma membrane. This accumulation of β-arrestin2-GFP is qualitatively similar to what we have previously reported for 10 μM dopamine-stimulated D3R in the presence of dynamin1-K44A (3). These results suggest that GRKs change the relative compartmentalization of the D₃R with β-arrestins when agonist is absent either by reducing the complement of β-arrestins prebound to the D₃R or shifting the equilibrium distribution of β-arrestin/D₃R toward CCPs. Consequently, other proteins that are also basally associated with D₃R may be affected by changes in GRK activity.

**β-Arrestin2 Interacts with the D₃R-associated Filamin**—The agonist-independent localization of β-arrestins with D₃Rs suggests that proteins basally binding D₃Rs are probable β-arrestin binding partners. This was tested in COS-7 cells for filamin A, which is localized in plasma membrane lipid rafts and is known to be important for the regulation of D₃R signaling in cells (15). COS-7 cells in comparison with other cell lines, such as HEK-293 cells, express relatively low levels of endogenous β-arrestins and thus provide a better background in which to assess transfected arrestin interactions by immunological methods (29). An association under basal conditions between β-arrestin2 and filamin A was confirmed by Western blot of COS-7 cell immunoprecipitates obtained using FLAG-tagged β-arrestin2 and anti-FLAG antibodies (Fig. 3A).

There are three human filamin isotypes (A, B, and C). They are ~70% homologous and structurally similar, consisting of multiple (twenty-four) Ig-like repeats that extend through their C termini (30). Their degree of homology and functional redundancy (30) suggests they all are potential β-arrestin binding partners. Indeed, a yeast two-hybrid library screen in an attempt to identify β-arrestin2-interacting proteins using the first 80 amino acids of the β-arrestin2 N terminus as bait yielded two colonies containing the distal third of human filamin C. The ability of full-length β-arrestin2 to also interact with this filamin segment is shown in Fig. 3B.

As observed above, the basal association of β-arrestin with filamin suggests that filamin may also be subject to GRK regulation. If so, D₃R signaling could be dramatically affected by changes in GRK activity directed toward destabilizing filamin/receptor association. To investigate this question, we began by studying the general relationship between filamin and D₃R signaling in cells that do not normally express filamin A.

**Filamin Expression Regulates the Coupling of the D₃R to G Protein**—The importance of filamin to D₃R second messenger signaling can be established using M2 cells, which normally do not express filamin A (15, 16). First, we are able to transfet M2 cells with quantitatively similar amounts of filamin A as contained in A7 cells (Fig. 4A). Although the total amounts of expressed immunoreactive filamin in the cell populations are similar, immunostaining and GFP co-transfection demonstrated that only one-third to one-half of the M2 cells are transfected and the efficiency of co-transfection, although appreciable, is less than 100% (data not shown).

We investigated the relationship between filamin A and G protein signaling of the D₃R in M2 cells by measuring GTPγS binding in the presence of increasing concentrations of DA (Fig. 4B). In order to improve the sensitivity of the assay, GTPγS binding was measured in M2 and control A7 cells transfected with the α subunit of G protein (Goα) in addition to D₃R. With coupling efficiency defined as the number of GTPγS molecules bound to the α subunit of G protein per receptor molecule, the basal G protein coupling of the D₃R was 9.4 ± 0.4% in M2 cells and 19.2 ± 1.7% in the control A7 cells, which express filamin A (Fig. 4B) (21). Co-expression of filamin A in M2 cells (M2-Filamin A) increased G protein coupling of D₃R to levels more nearly equivalent to those observed in A7 cells (Fig. 4B, middle curve). D₃R-S309A, a D₃R mutant that has a point mutation in an amino acid important for filamin A binding (15), exhibited reduced coupling efficiency (Fig. 4C). These results indicate a role for filamin A in D₃R-G protein signaling and suggest that alteration of filamin A/D₃R association would be one means to rapidly change D₃R signaling potential.

**Agonist Treatment Reduces the Association between the D₃R and Filamin A**—Filamin A interacts with a motif in the D₃R third cytoplasmic loop (14, 16), and a decrease in the cellular content of filamin A reduces, but does not totally prevent, D₃R signaling (Fig. 4, B and C). As such, receptors may retain the capacity to bind arrestins in the absence of filamin, and this is demonstrated by the immunoblot in Fig. 4D, where β-arrestin2 co-immunoprecipitated with FLAG-D3R in M2 cells. However, a dynamic reduction in the interaction between D₃R and fil-
GRK Regulates a D₃R, Filamin, β-Arrestin Complex

Fig. 4. Effects of filamin A on D₃R-G protein coupling. A, the ability to express filamin in M2 cells by electroporation was assessed 48 h post-transfection by immunoblotting using a monoclonal anti-filamin antibody. Lanes 1 and 5 served as controls, and 5 μg of filamin A cDNA or control vector was electroporated in the presence of 10 μg of D₃R cDNA at 300 V (lane 2), 320 V (lane 3), or 240 V (lane 7) and 1000 microfarads. Equal amounts of protein were added to each lane. Relative quantitation of each filamin band in the lanes after subtracting background was as follows: 0, 45, 59, 72, and 88 for lanes 1, 2, 3, 5, and 7, respectively. B, the effects of filamin A on GTPγS binding were evaluated in M2 and A7 cells over a range of dopamine concentrations. Cells were transfected with 10 μg of D₃R-pCMV5, 5 μg of Goα-pCMV5, with or without 5 μg of filamin A in pCDNA by electroporation (240 V/1000 microfarads for A7 cells and 320 V/1000 microfarads for M2 cells). The expression levels for M2-D₃R, M2-Fil-D₃R, and A7-D₃R were 1.09, 1.17, and 1.53 pmol/mg protein, respectively. Equal numbers of receptors were added per tube. Data represent results from two independent experiments with similar outcomes. C, HEK-293 cells were transfected with 3 μg of cDNAs encoding wild type D₃R or D₃R-S309A and 2 μg of Goα-pCMV5. The receptor expression level was 1.16 and 0.95 pmol/mg protein, respectively. Equal receptor amounts were added per tube. D, M2 and A7 cells transfected with β-arrestin2 and FLAG-tagged D₃R were assessed for whether the two proteins could be co-immunoprecipitated using anti-FLAG antibody in the absence (M2 cells) and presence (A7 cells) of endogenous filamin. E, effects of agonist stimulation on the interaction between D₃R and filamin A were studied in COS-7 cells transfected with 3 μg of FLAG-D₃R and 2 μg of filamin A. After 48 h, cells were washed with prewarmed Dulbecco’s modified Eagle’s medium and treated with 30 nM dopamine dissolved in Dulbecco’s modified Eagle’s medium containing 10 μM sodium metabisulfite for 5 min. Immunoprecipitates were analyzed by SDS-PAGE, and immunoblots were probed with antibodies for filamin A at 1:1,000 dilutions. IP, immunoprecipitation.

Exogenous GRK Expression Reduces the Basal Association between D₃R and Filamin A—GRK activity increases secondarily to agonist activation of GPCRs. Therefore, we tested whether the ability of agonists to dissociate filamin A-receptor complexes could be reproduced by enhancing GRK activity without exposing receptors to agonist. In COS-7 cells, the co-expression of GRK2 or GRK3 reduces the basal interaction between D₃R and filamin A (Fig. 5A, upper panel, compare lane 3 versus lane 4 and both lanes 3 and 4 versus lane 2) as demonstrated by immunoprecipitation of the FLAG-tagged receptor and immunoblotting for filamin A. Additionally, as discussed in Fig. 2B, GRK co-expression reduces the basal association of D₃R with β-arrestins (Fig. 5A, lower panel, compare lane 3 and lane 4 and lane 3 versus lane 2). In contrast, a kinase-dead, dominant negative GRK2 (GRK2-K220R) enhances the basal association of the D₃R with filamin A and that of the D₃R with β-arrestin (Fig. 5B, compare lane 2 versus lane 3). These effects of GRK activity on the interactions between the D₃R, filamin A, and β-arrestin were not direct results of GRK2 phosphorylation of filamin A. In other words, purified filamin A was not phosphorylated by purified GRK2 (data not shown).

GRK Expression Reduces D₃R Signaling—Since an association between the D₃R and filamin A is needed for efficient G protein coupling, effects of GRK expression on the G protein coupling and the inhibition of cAMP production were tested. As expected from the results in Fig. 5, A and B, GRK overexpression significantly reduced both the basal and agonist-induced G protein coupling of the D₃R as assessed by GTPγS binding (Fig. 5C). The GTPγS results indicate that second messenger production should be inhibited less well with excess GRK expression (i.e., the D₃R-mediated decrease in cellular cAMP content should be smaller). To test the effects of GRKs on the ability of the D₃R to inhibit cAMP production, the cellular cAMP was
first raised by forskolin stimulation, and the D₃R-mediated decrease in cellular cAMP was determined with increasing concentrations of quinpirole in cells transiently expressing GRK2 or GRK3. The dose-response curves in the presence of added kinase exhibit a decrease in \( V_{\text{max}} \) and an increase in IC₅₀ (i.e. a shift of the curve to the right) (Fig. 5D). The IC₅₀ (nM) and the maximal inhibition (%) for the control (0.31 ± 0.09 nM and 70 ± 7% (p < 0.05)) is shifted to 0.58 ± 0.15 nM and 55 ± 3% for GRK2 and to 0.53 ± 0.01 nM and 49 ± 3% (p < 0.05) for GRK3.

**DISCUSSION**

Recent studies on the distribution of D₃R protein in the brain show that it is expressed on virtually all dopaminergic neurons (4, 5), strongly supporting the idea of the D₃R as an autoreceptor. In agreement with this autoreceptor hypothesis, only a negligible fraction of plasma membrane D₃R internalizes as a result of persistent DA stimulation, even with increased GRK activity, so that the plasma membrane population of D₃Rs remains relatively constant (3). Therefore, GRK/β-arrestin-directed receptor internalization in CCPs plays a relatively minor role in the short term, agonist-mediated D₃R desensitization process, whereas GRK regulation of D₃R plasma membrane compartmentalization may play a more significant role.

Our studies indicate that GRKs serve as rheostats for adjusting the agonist-mediated G protein signaling potential of D₃Rs that are in basal conformations. The D₃R possesses an extensive third intracellular loop that contains multiple, putative GRK phosphorylation sites surrounding and dispersed throughout the filamin binding domain (14, 16). Our findings indicate that basal GRK phosphorylation of these sites potentially reduces D₃R binding to filamin and greatly reduces G protein-mediated signaling. Thus, basal GRK activity through phosphorylation of the D₃R third loop may simultaneously determine both the number of D₃R signaling complexes in filamin-rich lipid rafts and the extent of their agonist-mediated signaling, since agonist binding appears to shift receptors away from an association with filamin to one with β-arrestin.

In this novel GPCR desensitization model, basal GRK activity controls formation of D₃R/β-arrestin-filamin complexes, raising the question as to why arrestins need to co-localize with receptors and filamin at this stage. A general and perhaps the most basic explanation may be that arrestins are co-localized because this region is where signaling occurs and where homologous desensitization should occur most expeditiously. An explanation for the D₃R in particular may lie in the role D₃Rs presumably fill as autoreceptors. In order to provide continuous monitoring of ligand concentrations, D₃Rs should be more
resistant to removal from the cell surface than receptors without this functional requirement. As such, the D3Rs exhibit relatively limited ability to remain associated with arrestins in comparison with other GPCRs, which is indicative of an intrinsically lower affinity. Therefore, from mass action considerations, the local concentration of arrestins plays a much greater role in sustaining D3R desensitization than it would for a receptor with intrinsically greater arrestin affinity. Moreover, induced changes in the local concentration of β-arrestins with D3Rs that are reciprocal to changes in GRK activity may provide a negative feedback for maintaining receptor signaling potential relatively constant. It would be interesting to test whether this novel model of GPCR desensitization will be applicable to other autoreceptor GPCRs that are involved in neurotransmitter concentration-dependent regulation of secretion.

Overall, D3R signaling may be desensitized by at least two GRK-mediated mechanisms. Desensitization occurs with a reduction in G protein coupling due to an inability of filamin to localize receptors near G proteins. Alternatively, desensitization may occur from a potentially reversible, direct blockade of G protein coupling by arrestin/receptor binding. Only profound enhancements in GRK activity can marginally sustain a more classical β-arrestin/receptor desensitization picture involving clathrin-mediated receptor endocytosis.

An observation indicating that a modulation of basal GRK activity could be therapeutically useful for DA-related illness stems from studies of GRK6 knock-out mice. These animals become hyperresponsive to DA stimulation (31), indicating a role of GRK6 in regulation of postsynaptic D2-like receptors. Additionally, the GRK6 knock-out mouse striatal membranes display similarly increased GTPγS binding relative to wild type mouse controls, as do our control cells compared with cells expressing additional GRK2 or -3. Interestingly, we observed that GRK3-KO mice are hyposensitive to DA stimulations in locomotor assays (20), a finding that would be consistent with a specific role of this GRK in the regulation of DA autoreceptor function. Thus, if a genetic reduction of GRK activity can enhance dopamine receptor signaling in vivo, then a corresponding type-specific GRK inhibitor/activator might facilitate responsiveness to suboptimal levels of endogenous dopamine in the treatment of movement disorders like Parkinson’s disease or to other monoaminergic neurotransmitters such as serotonin in the treatment of mood disorders like depression.

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REFERENCES
1. Joseph, J. D., Wang, Y. M., Miller, P. B., Budgin, E. A., Picetti, R., Gainetdinov, R., R., Caron, M. G., and Wightman, R. M. (2002) Neuroscience 112, 39–49
2. Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998) Physiol. Rev. 78, 189–225
3. Kim, K. M., Valenzano, K. J., Robinson, S. R., Yao, W. D., Barak, L. S., and Caron, M. G. (2001) J. Biol. Chem. 276, 37408–37414
4. Dzah, J., Pilon, C., Le Foll, B., Gros, C., Triller, A., Schwartz, J. C., and Sokoloff, P. (2000) J. Neurosci. 20, 8677–8684
5. Gurevich, E. V., and Joyce, J. N. (1999) Neuropsychopharmacology 20, 60–80
6. Jones, S. R., Gainetdinov, R. R., Hu, X. T., Couper, D. C., Wightman, R. M., White, F. J., and Caron, M. G. (1999) Nat. Neurosci. 2, 649–655
7. Luttrell, L. M., and Lefkowitz, R. J. (2002) J. Cell Sci. 115, 455–465
8. Ferguson, S. S. (2001) Pharmacol. Rev. 53, 1–24
9. Kim, O. J., Gardner, B. R., Williams, D. B., Marine, S. P., Cabrera, D. M., Peters, J. D., Mok, C. C., Kim, K. M., and Sibley, D. K. (2004) J. Biol. Chem. 279, 7999–8010
10. Oakley, R. H., Laporte, S. A., Holt, J. A., Barak, L. S., and Caron, M. G. (2001) J. Biol. Chem. 276, 19452–19460
11. McDonald, P. H., Chow, C. W., Miller, W. E., Laporte, S. A., Field, M. E., Lin, F. T., Davis, R. J., and Lefkowitz, R. J. (2000) Science 290, 1574–1577
12. Oakley, R. H., Laporte, S. A., Holt, J. A., Barak, L. S., and Caron, M. G. (1999) J. Biol. Chem. 274, 32249–32257
13. Krueger, K. M., Daaka, Y., Pitcher, J. A., and Lefkowitz, R. J. (1997) J. Biol. Chem. 272, 5–8
14. Lin, R., Karp, K., Kabbani, N., Goldman-Rakic, P., and Levenson, R. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 5258–5263
15. Li, M., Li, C., Weingarten, P., Bunzow, J. R., Grandy, D. K., and Zhou, Q. Y. (2002) Biochem. Pharmacol. 65, 859–863
16. Li, M., Bermak, J. C., Wang, Z. W., and Zhou, Q. Y. (2000) Mol. Pharmacol. 57, 446–452
17. Enz, R. (2002) FEBS Lett. 514, 184–188
18. Seck, T., Barney, R., and Horre, W. C. (2003) J. Biol. Chem. 278, 10408–10416
19. Onopriashvili, I., Andria, M. L., Kramer, H. K., Ancevska-Taneva, N., Miller, V. M., and Simon, E. J. (2003) Mol. Pharmacol. 64, 1092–1100
20. Gainetdinov, R. R., Premont, R. T., Bohn, L. M., Lefkowitz, R. J., and Caron, M. G. (2004) Annu. Rev. Neurosci. 27, 107–144
21. Cunningham, C. C., Gorlin, J. B., Kwiatkowski, D. J., Hartwig, J. H., Janney, P. A., Byers, H. R., and Stossel, T. P. (1992) Science 255, 2325–2327
22. Okita, Y., Suzuki, N., Nakatani, S., Hartwig, J. H., and Stossel, T. P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2122–2128
23. Robinson, S. W., and Caron, M. G. (1996) J. Neurochem. 67, 212–218
24. Barak, L. S., Ferguson, S. S., Zhang, J., and Caron, M. G. (1997) J. Biol. Chem. 272, 27497–27500
25. Barak, L. S., Oakley, R. H., Laporte, S. A., and Caron, M. G. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 93–98
26. Freedman, N. J., Liggett, S. B., Drachman, D. E., Pei, G., Caron, M. G., and Lefkowitz, R. J. (1995) J. Biol. Chem. 270, 17953–17961
27. Laporte, S. A., Oakley, R. H., Zhang, J., Holt, J. A., Ferguson, S. S., Caron, M. G., and Barak, L. S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3712–3717
28. Amlaky, N., Kilpatrick, B. F., and Caron, M. G. (1984) FEBS Lett. 176, 436–440
29. Menard, L., Ferguson, S. S., Zhang, J., Lin, F. T., Lefkowitz, R. J., Caron, M. G., and Barak, L. S. (1997) Mol. Pharmacol. 51, 800–808
30. van der Flier, A., and Sonnenberg, A. (2001) Biochim. Biophys. Acta 1538, 99–117
31. Gainetdinov, R. R., Bohn, L. M., Sotsikova, T. D., Cyri, M., Laakso, A., Macrae, A. D., Torres, G. E., Kim, K. M., Lefkowitz, R. J., Caron, M. G., and Premont, R. T. (2003) Neuron 38, 291–303