Phosducin, which tightly binds βγ-subunits of heterotrimeric G-proteins, has been conjectured to play a role in regulating second messenger signaling cascades, but to date its specific function has not been elucidated. Here we demonstrate a potential role for phosducin in regulating olfactory signal transduction. In isolated olfactory cilia certain odorants elicit a rapid and transient cAMP response, terminated by a concerted process which requires the action of two protein kinases, protein kinase A (PKA) and a receptor-specific kinase (GRK3) (Schleicher, S., Boekhoff, I. Arriza, J., Lefkowitz, R. J., and Breer, H. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1420–1424). The mechanism of action of GRK3 involves a Gβγ-mediated translocation of the kinase to the plasma membrane bound receptors (Pitcheer, J. A., Ingles, J., Higgins, J. B., Arriza, J. L., Casey, P. J., Kim, C., Benovic, J. L., Kwatra, M. M., Caron, M. G., and Lefkowitz, R. J. (1992) Science 257, 1264–1267). A protein with a molecular mass of 33 kDa that comigrates on SDS gels with recombinant phosducin and which is immunoreactive with phosducin antibodies is present in olfactory cilia. Recombinant phosducin added to permeabilized olfactory cilia preparations strongly inhibits termination of odorant-induced cAMP response and odorant-induced membrane translocation of GRK3. In addition, the cAMP analogue dibutylryl cAMP stimulates membrane targeting of the receptor kinase. This effect is presumably due to PKA-mediated phosphorylation of phosducin, which diminishes its affinity for binding to the Gβγ-subunit, thereby making Gβγ available to function as a membrane anchor for GRK3. A specific PKA inhibitor blocks the odorant-induced translocation of the receptor kinase. Consistent with this formulation, a non-phosphorylatable mutant of phosducin (phosducin Ser-73 → Ala) is an even more effective inhibitor of desensitization and membrane targeting of GRK3 than the wild-type protein. A phosducin mutant that mimics phosphorylated phosducin (phosducin Ser-73 → Asp) lacks this property and in fact recruits GRK3 to the membrane and potentiates desensitization. These results suggest that phosducin may act as a phosphorylation-dependent switch in second messenger signaling cascades, regulating the kinetics of desensitization processes by controlling the activity of Gβγ-dependent GRKs.

The olfactory system responds precisely to iterative stimulation; this characteristic feature is due to the phasic responses of receptor cells (3), based on a rapid termination of the odor-induced primary reaction (4). Recent studies have indicated that olfactory signaling is terminated by uncoupling the transduction cascade; the second messenger signal elicited by odors is turned off by a negative feedback reaction controlled by phosphorylation of odorant receptors mediated by two types of enzymes, a second messenger controlled kinase and a receptor specific kinase (GRK3) (1, 5–8).

These observations raise the possibility that the two kinases act sequentially in a reaction cascade that is initiated by activating the second messenger-dependent kinase. The details of how these kinases interact, however, remain elusive. Several mechanisms seem plausible; second messenger-dependent kinase might directly phosphorylate and thus increase the enzymatic activity of receptor-specific kinases, similar to kinase-mediated activation of enzymes in regulating metabolic pathways (9). However, there is no evidence for this mechanism; in fact, the sequence of GRK3 does not contain any consensus site for PKA phosphorylation. Alternatively, the receptor-specific kinase may be under tonic control of an as yet unknown inhibitor, the phosphorylation of which by PKA may relieve this inhibitory constraint (1, 10).

It has recently been found that interaction of GRKs with βγ-subunits of heterotrimeric G-proteins (Gβγ-subunits) is required for the cytosolic GRKs to be translocated to the membrane and subsequently phosphorylated the agonist-occupied receptors (2, 11–14). These findings were extended by the observation that membrane targeting of GRK by docking onto βγ-subunits is an essential prerequisite for turning off the second messenger cascade (15).

Phosducin, a major soluble phosphoprotein first discovered in mammalian retinal tissue (16, 17) also binds βγ-subunits of heterotrimeric G-proteins (18) probably via structural domains within the amino-terminal region which are homologous to the Gβγ-binding domain of GRKs (19). The notion that both proteins may compete for binding sites of the Gβγ complex has recently been confirmed (10). Moreover, phosducin is readily phosphorylated at serine residue 73 by protein kinase A, and this modification significantly reduces its binding affinity for Gβγ-subunits (19–21). In this study we set out to explore the hypothesis that phosducin might be capable of serving as a PKA controlled inhibitor of GRK in olfactory cilia.

**EXPERIMENTAL PROCEDURES**

**Materials**

Sprague-Dawley rats were purchased from Charles River, Sulzfeld; fresh bovine olfactory epithelium was obtained from a local slaughterhouse and stored at –70 °C.

The abbreviations used are: GRK3, receptor-specific kinase; MOPS, 4-morpholinepropanesulfonic acid; PKA, protein kinase A.
FIG. 1. Detection of phosducin in isolated olfactory cilia preparations. 2.5 mg of cytosolic protein from bovine isolated olfactory cilia were immunoprecipitated with affinity purified rabbit anti-phosducin antibodies. The immunoprecipitates were resolved on SDS-polyacrylamide gel electrophoresis, blotted, and probed with anti-phosducin antibodies (IgGs, 1:5000) raised in goat (lane 3). A heat-inactivated cytosolic aliquot from olfactory cilia was used as a control (lane 2); 200 ng of recombinant phosducin was used as a standard (lane 1). Molecular mass of standards, shown in kilodaltons, are indicated.

Methods

Preparation of Phosducin Mutants—A human phosducin plasmid (pBS-33K) was kindly provided by Dr. T. Shinohara (National Institutes of Health). An MutI site (ACCGGT) was introduced into pBS-33K by a single base mutation at position 252 (A → C) employing a mutagenic reverse primer containing a BamH site. The forward primer contained a sequence complementary to the 5'-end of phosducin (including the start codon and 5'-nucleotides) and contained a 5'-KpnI site. The polymerase chain reaction fragment generated from these primers was subcloned into pBS-33K and digested with KpnI/BamHI. Polymerase chain reaction-cassette mutagenesis of Ser-73 to Ala and Asp was accomplished using this new construct and the flanking MutI/BamHI sites. The introduction of mutations was verified by sequencing with primers containing appropriate restriction sites. The mutant phosducin DNAs were ligated into the pQE30 vector (Qiagen) as a fusion protein with 10 amino acids containing a hexahistidine sequence. Fusion protein constructs were introduced into the Escherichia coli strain BL21 and induced as described previously (23, 24).

Isolation of His-6-Fusion Proteins—The induced cells were lysed in 50 mM HEPES, 1% Tween 20, protease inhibitor mixture (Boehringer Mannheim), pH 7.5, by freeze-thawing. The lysed cells were centrifuged at 15,000 rpm (SS-34 rotor, Sorvall instruments) for 20 min at 4 °C, and the pellet was resuspended in buffer A (50 mM HEPES, 300 mM NaCl, pH 7.5) containing 8 mM urea. After 2 h incubation with rotation, the solution was centrifuged at 15,000 rpm for 20 min. Ni-resin (ProBond; Invitrogen) was then added to the supernatant and incubated at 4 °C for 1 h with rotation. The same volume of buffer A containing 4 mM urea was added, and the beads were collected in a column. The beads were washed with buffer A containing 4, 3, 2, and 1 mM urea stepwise, and the washed beads were incubated with buffer A containing 1 mM urea overnight at 4 °C. The refolded His-6-fusion proteins were eluted with buffer A containing 500 mM imidazole. The eluate was then dialyzed against 20 mM HEPES, 350 mM NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, pH 7.5, for 2 h at 4 °C. The protein concentration was determined by Bradford method (Bio-Rad).

Isolation of Olfactory Cilia—Enriched preparations of olfactory cilia from rat and bovine olfactory epithelium were isolated using the calcium-shock method described by Anholt et al. (25) and Chen et al. (26). All isolation steps were performed at 4 °C. The olfactory epithelium was washed in Ringer solution (120 mM NaCl, 5 mM KCl, 1.6 mM KH2PO4, 25 mM NaHCO3, 7.5 mM glucose, pH 7.4) and subsequently transferred to Ringer solution containing 10 mM calcium. Detached cilia were separated by centrifugation for 5 min at 7,700 × g. The supernatant was collected, and the resulting pellet was resuspended again in Ringer solution with 10 mM CaCl2 and centrifuged as described above. The supernatants were combined and centrifuged for 15 min at 27,000 × g. The pellet containing the detached cilia was resuspended in TME buffer (10 mM Tris/Cl, 3 mM MgCl2, 2 mM EGTA, pH 7.4) and stored at −70 °C.

Immunoprecipitation and Western Blot Analysis—Phosducin was immunoprecipitated from bovine isolated olfactory cilia preparations as described recently (27). Briefly, cytosolic fractions were prepared by homogenizing 8 mg of isolated cilia under liquid nitrogen and suspending them in 20 mM Tris, 5 mM EDTA, 5 mM EGTA, 20 mM/liter benzamidine, 20 mM phenylmethylsulfonyl fluoride, pH 7.4. After thawing, the samples were centrifuged for 30 min at 50,000 × g, and the resulting supernatant was incubated for 1 h at 4 °C with pre-washed Protein A-Sepharose (Pharmacia Biotech Inc.). After an additional centrifugation step, 5 μg of affinity purified rabbit anti-phosducin antibody was added to the supernatant and incubated for 2 h before the immune complexes were bound by adding another aliquot of Protein A-Sepharose and pelleted by centrifugation. After washing the pellets with Brij buffer (1% Brij 96, 50 mM NaCl, 50 mM Tris/Cl, 10 mM EDTA, 10 mM EGTA, 20 mM/liter benzamidine, 20 mM phenylmethylsulfonyl fluoride, pH 7.4) the samples were resuspended in SDS-loading buffer containing 6 mM urea and heated to 95 °C for 5 min to release the protein. As a control, the same procedure was done with cytosol that has been heated to 95 °C for 5 min followed by pelleting of the denatured protein. The samples were separated on SDS-polyacrylamide gels, and phosducin was detected using the IgG fraction of a second phosducin antiserum raised in goats (22) and the enhanced chemiluminescence system (Amersham Corp.).

Determination of Odor-induced Second Messenger Responses—A rapid kinetic system was used to determine odorant-induced changes of second messenger concentrations in the subsecond time range. Stimu-
loration experiments were performed at 37 °C as described previously (4). Syringe I contained the stimulation buffer (200 mM NaCl, 10 mM EGTA, 50 mM MOPS, 2.5 mM MgCl₂, 1 mM dithiothreitol, 0.05% sodium cholate, 1 mM ATP, and 2 μM GTP, pH 7.4) and 12 mM free calcium calculated and adjusted as described by Pershadsingh and McDonald (28). Syringe II contained the olfactory cilia, and syringe III was filled with stop solution (7% perchloric acid). If not indicated otherwise, 205 μl of stimulation buffer was mixed with 20 μl of cilia; at the appropriate time (ms) the reaction was stopped by injection of perchloric acid. Quenched samples were stored on ice for 20 min and then analyzed for second messenger concentrations as described previously (29).

In all cases cilia were preincubated 10 min on ice with the recombinant phosducin mutants or the other modulators; control samples were incubated with TME buffer, which was used to dilute the protein. The concentrations of the different modulators indicated are the concentrations during pretreating the cilia.

**Enzyme-linked Immunosorbent Assay—Odor-induced translocation of GRK3 from the cytosol to the membrane.** Isolated olfactory cilia preparations were incubated with 390 nM recombinant phosducin for 15 min; control samples were incubated with TME medium. After stimulation with an odorant mixture containing citralva, hedione, and eugenol (5 μM each) the samples were immediately fractionated. The resulting pellets and supernatants were assayed for GRK3 immunoreactivity employing subtype-specific antibodies as described previously (Boekhoff et al. (15)). The immunoreactivity of GRK3 in the soluble and particulate fractions was determined using aliquot samples. The sum of immunoreactivity in both fractions was taken as 100% and the percentage of each fraction calculated. The results are expressed in % of GRK3 immunoreactivity. Data are the means of three different experiments ± S.D.

**FIG. 3.** Phosducin prevents stimulus-induced translocation of GRK3 from the cytosol to the membrane. Isolated olfactory cilia preparations were incubated with 390 nM recombinant phosducin for 15 min; control samples were incubated with TME medium. After stimulation with an odorant mixture containing citralva, hedione, and eugenol (5 μM each) the samples were immediately fractionated. The resulting pellets and supernatants were assayed for GRK3 immunoreactivity. Data are the means of three different experiments ± S.D.

**FIG. 4.** Translocation of GRK3 to the membrane is induced by exogenous cAMP. Olfactory cilia preparations were pretreated with different concentrations of dibutyryl cAMP (1 μM; 100 μM), subsequently incubated with reaction buffer with or without an odorant mixture (citralva, hedione, eugenol, each 1 μM), and separated into cytosolic and membrane fractions, which were assayed for GRK3 immunoreactivity. The total immunoreactivity in both fractions was taken as 100%; the results are expressed as percentage of GRK3 immunoreactivity in the membrane fraction. Data are the means of three different experiments ± S.D.

In order to verify if phosducin is indeed present in olfactory cilia preparations, a combined immunoprecipitation/Western blot analysis was performed using specific antibodies as described previously (27). As shown in Fig. 1, a strong immunoreactive band of 33 kDa (lane 3) which comigrates with recombinant phosducin (lane 1) is visualized in the olfactory cilia preparation. The specificity of the reaction is supported by the negative reaction in lane 2 (control), which represents a boiled sample of cilia. Based on the labeling intensity, the concentration of phosducin in the cilia preparation seems to be in the same range as in other tissues (27).

To approach the question of whether phosducin may affect the kinetics of odor-induced second messenger signaling, the effect of exogenous phosducin on odor-induced cAMP responses...
in olfactory cilia was analyzed. Cilia preparations from the rat olfactory epithelium were preincubated with recombinant phosducin and subsequently stimulated with a mixture of odorants (citral, hedione, and eugenol, each 5 µM) and then stimulated with a mixture of odorants (citral, hedione, and eugenol, 1 mM each). The odorant-induced cAMP response was monitored in the subsecond time range. Stimulation of control samples elicited a rapid and transient increase in the concentration of cAMP (Fig. 2) as described previously (4). In samples pretreated with recombinant phosducin the “onset kinetics” of the odorant-induced cAMP signal were virtually unaffected; however, the “off kinetics” were significantly changed; the elevated cAMP level decayed with a much slower rate. These results indicate that exogenous phosducin inhibited the rapid termination of the odor-induced second messenger signal; thus phosducin seems to interfere with the desensitization process.

Since membrane targeting of GRK3 is a prerequisite for terminating the olfactory signaling cascade (15), we investigated if exogenous phosducin may affect the odor-induced translocation of GRK3. Isolated olfactory cilia were preincubated with exogenous phosducin and stimulated with an odor mixture; subsequently membrane and cytosolic fractions were separated and analyzed for receptor-specific kinase immunoreactivity as described previously (15). In untreated cilia prior to odorant stimulation, only 21.2 ± 3.6% of the GRK immunoreactivity was found in the membrane fraction and about 78.8 ± 3.6% was in the cytosol (Fig. 3). Stimulation with odorants induced a significantly different distribution pattern of the GRK immunoreactivity, cytosol (54.6 ± 4.6) and membrane fraction (45.3 ± 4.6). In contrast, when cilia preincubated with phosducin were stimulated with odorants, the distribution pattern of kinase immunoreactivity did not change; most of the GRK remained in the cytosolic fraction. These results imply that exogenous phosducin prevents the translocation of GRK3 to the membrane-anchored Gβγ and that this may be the critical step by which phosducin interferes with the rapid desensitization (Fig. 2).

If phosducin is tonically inhibiting the desensitization process by preventing GRK3 from docking onto Gβγ-subunits, then its phosphorylation by PKA may release this constraint, since previous studies have shown that phosphorylation of phosducin by PKA diminishes its potential to interfere with the interaction of GRK with Gβγ (10, 19). To assess the relevance of this hypothesis, experiments were performed measuring membrane targeting of GRK3 in the presence of a cAMP analogue which should activate protein kinase A, leading to phosphorylation of endogenous phosducin.

The data indicate that in the presence of elevated cAMP levels, a significantly higher portion of GRK3 was associated with the membrane (Fig. 4). These results support the notion that protein kinase A may play a key role in controlling the membrane targeting of GRK3 in olfactory cilia induced by "cAMP odorants."

This view was further scrutinized in experiments using a specific inhibitor of protein kinase A. As shown in Fig. 5, the translocation of GRK3 elicited by odorant stimulation is attenuated by the Walsh inhibitor in a dose-dependent manner.

![Graph](image-url)
These results further substantiate the essential role of PKA-mediated phosphorylation reactions in regulating the translocation of GRK.

To further approach the functional role of phosphorylated and non-phosphorylated phosducin, distinct mutants were prepared. In the phosducin-Ser-73 → Ala mutant, serine 73 was changed to alanine, thus preventing its PKA-mediated phosphorylation; the phosducin-Ser-73 → Asp mutant, in which serine 73 was substituted by aspartic acid, should mimic the phosphorylated form of phosducin due to its negatively charged side chain. Analyzing each mutant for its capability to become phosphorylated by PKA revealed that only wild-type phosducin undergoes phosphorylation whereas both mutants (Ala, Asp) are not phosphorylated by PKA (Fig. 6A). These results confirm previous observations indicating that serine 73 is the target site for phosphorylation of phosducin by PKA (21).

To characterize the functional properties of the different phosducin isoforms, their binding capacity for βγ-subunits of trimeric G-proteins was assessed. As shown in Fig. 6B, in control samples (without fusion protein) there is no βγ binding detectable; the wild-type phosducin as well as the phosducin-Ala mutant interacted with βγ-subunits to a similar extent. The pseudophosphorylated phosducin-Asp mutant displayed a significantly reduced binding to βγ-subunits, probably due to its reduced affinity.

Subsequently the mutated forms of phosducin were assessed for their potency to affect termination of odor-induced second messenger signaling. The phosducin-Ala mutant, which cannot be modified by PKA phosphorylation (Fig. 6A) and which efficiently interacts with Gβγ-subunits (Fig. 6B), prevented the rapid decay of the odor-induced second messenger response (Fig. 7A); interestingly, this non-modifiable phosducin form was even more effective than the native form (Fig. 2) suggesting that exogenously applied native phosducin may be partially inactivated by PKA phosphorylation during preincubation.

In cilia preparations preincubated with the pseudo-phosphorylated mutant phosducin-Asp, the rapid termination of the odor-induced cAMP signal was not blocked, but rather the cAMP signal decayed even more rapidly (Fig. 7B); in addition, the intensity of the response was reduced. These results indicate that phosducin-Asp does not attenuate but rather seems to facilitate the desensitization process. This could mean that an excess of pseudo-phosphorylated phosducin may expose more βγ-subunits for GRKs targeting, thus causing a recruitment of GRKs. Therefore, it is conceivable that an excess of phosphorylated phosducin may allow a membrane docking of GRK even before receptor stimulation.

To assess this notion, the GRK3 distribution in cilia preparations was determined in the presence of the two phosducin mutants. As can be seen in Fig. 8A, the phosducin-Ala completely blocked the odor-induced translocation of GRK3 from the cytosol to the membrane (Fig. 8A). Thus, the non-phosphorylated form of phosducin, which actively interferes with the desensitization process (Fig. 7A) and efficiently interacts with Gβγ-subunits (Fig. 6A), prevents odor-induced translocation of GRK3.

To approach the properties of the phosphorylated form of phosducin on GRK3 distribution, cilia were incubated with phosducin-Asp but not stimulated with odorants (Fig. 8B). The results indicate that after preincubation with phosducin-Asp 34.7 ± 9.8% of the GRK3 immunoreactivity was located in the membrane fraction compared with 21.2 ± 3.6 in untreated cilia. Upon stimulation with odorants under the same conditions, the proportion of GRK bound to the membrane (44.6 ± 8.2%) increased only slightly. These results indicate that an excess of pseudo-phosphorylated phosducin elicits a membrane targeting of GRK3.
DISCUSSION

Experiments using exogenous phosducin and its mutated forms are consistent with the hypothesis that phosducin may control the kinetics of second messenger signaling in olfactory cilia by governing the stimulus-dependent translocation of a receptor-specific kinase. This observation is in line with previous in vitro studies demonstrating that binding of GRK2 to βγ-subunits is inhibited by phosducin (10). In addition, experiments employing phosducin mutants support the view that phosphorylation of phosducin may change its functional properties. A phosducin mutant, which cannot be phosphorylated, prevented the rapid decay of an odor-induced second messenger response even more efficiently than the native form. Furthermore, phosducin mutants that mimic the phosphorylated form, due to a negatively charged side chain, caused a constitutive translocation of GRK3 to the membrane suggesting that the phosphorylation status of phosducin may allow an anchoring of GRK3 to βγ-subunits. This could be due to the reduced binding affinity of phosphorylated phosducin to βγ-subunits as previously observed in experiments using fusion proteins with the amino terminus of phosducin (19, 31). Moreover, reconstitution experiments have shown that the competition of phosducin and GRK for a common binding site on the Gβγ-complex is antagonized following phosphorylation of phosducin by PKA (10).

Together with previous in vitro observations the findings of this study support a model in which phosducin is a phosphorylation-dependent regulator governing the membrane targeting of GRK; in its non-phosphorylated state phosducin strongly interacts with the βγ-subunits of trimeric G-proteins thus preventing the anchoring of GRK3 to these sites. Upon phosphorylation, the affinity of phosducin for the βγ-subunits is reduced and the blockade of GRK anchoring is relieved, so that the GRK can dock onto βγ-subunits and phosphorylate receptor proteins, thus uncoupling the transduction cascade.

Previous studies have shown that a sequential interplay of two types of kinases is involved in terminating the odor-induced second messenger signal, a second messenger controlled kinase and a receptor-specific kinase (GRK3) (1, 5–7); both types of kinases are required for an effective desensitization of the olfactory cascade (1). The sequential interplay of the two enzymes could be explained if phosducin is the previously proposed endogenous inhibitor of GRK3 action (15). An odorant-induced rise of cAMP levels would activate protein kinase A leading to phosphorylation of phosducin which triggers the membrane targeting of GRK3 and phosphorylation of the receptor protein.

Whether termination of the alternative olfactory pathway, odorant-induced generation of inositol 1,4,5-trisphosphate, is controlled by a similar cascade is unclear. Previous studies have indicated that in this case protein kinase C plays a critical role, analogous to kinase A in the cAMP pathway (5). The lack of obvious consensus sites for phosphorylation by protein kinase C makes it less likely that phosducin may also be involved in the phospholipase C pathway. However, recent studies have demonstrated that stimulation of protein kinase C elicits an activation of GRK due to a phosphorylation-mediated translocation to the plasma membrane (32).

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REFERENCES

1. Schleicher, S., Boekhoff, I. Arriza, J., Leffkowitz, R. J., and Breer, H. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 1420–1424
2. Pitcher, J. A., Inglese, J., Higges, J. B., Arriza, J. L., Casey, P. J., Kim, C., Benovic, J. L., Kwaatra, M. M., Caron, M. G., and Leffkowitz, R. J. (1992) Science 257, 1264–1267
3. Getchel, T. V., and Shepherd, G. M. (1978) J. Physiol. (London) 282, 541–560
Phosducin in Olfactory Signaling

4. Breer, H., Boekhoff, I., and Tareilus, E. (1990) Nature 345, 65–68
5. Boekhoff, I., and Breer, H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 471–474
6. Boekhoff, I., Schleicher, S., Strotmann, J., and Breer, H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11983–11987
7. Dawson, T. M., Arriza, J. L., Javarsky, D. E., Borisy, F. F., Attramadal, H., Lefkowitz, R. J., and Ronnett, G. V. (1993) Science 259, 825–828
8. Krieger, J., Raming, K., Strotmann, J., Wanner, I., Boekhoff, I., Schleicher, S., Geus, P., and Breer, H. (1994) Eur. J. Biochem. 219, 829–835
9. Cohen, P. (1986) Eur. J. Biochem. 151, 439–448
10. Hekman, M., Bauer, P., Schleemann, R., and Lohse, M. J. (1994) FEBS Lett. 343, 120–124
11. Haga, K., and Haga, T. (1990) FEBS Lett. 268, 43–47
12. Haga, K., and Haga, T. (1992) J. Biol. Chem. 267, 2222–2227
13. Kameyama, K., Haga, K., Haga, T., Kentani, K., Katada, T., and Fukada, Y. (1993) J. Biol. Chem. 268, 7755–7758
14. Inglese, J., Koch, W. J., Caron, M. G., and Lefkowitz, R. J. (1992) Nature 359, 147–150
15. Boekhoff, I., Inglese, J., Schleicher, S., Koch, W. J., Lefkowitz, R. J., and Breer, H. (1994) J. Biol. Chem. 269, 37–40
16. McGinnis, J. F., and Leveille, P. J. (1985) Curr. Eye Res. 4, 1127–1135
17. Lolley, R. N., Brown, B. M., and Farber, D. B. (1976) Anal. Biochem. 72, 248–254
18. Lee, R. H., Lieberman, B. S., and Lolley, R. N. (1987) Biochemistry 26, 3983–3990
19. Hawes, B. E., Touhara, K., Kurose, H., Lefkowitz, R. J., and Inglese, J. (1994) J. Biol. Chem. 269, 29825–29830
20. Kuo, C. H., Akiyama, M., and Miki, M. (1989) Mol. Brain Res. 6, 1–10
21. Lee, R. H., Brown, B. M., and Lolley, R. N. (1990) J. Biol. Chem. 265, 15860–15866
22. Schulz, K., Danner, S., Bauer, P., Schröder, S., and Lohse, M. J. (1996) J. Biol. Chem. 271, 22546–22551
23. Inglese, J., Luttrell, L. M., Iniguez-Lluhi, J. A., Touhara, K., Koch, W. J., and Lefkowitz, R. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3637–3641
24. Touhara, K., Inglese, J., Pitcher, J. A., Shaw, G., and Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 10217–10220
25. Anholt, R. R. H., Aebi, U., and Snyder, S. H. (1986) J. Neurosci. 6, 1403–1406
26. Chen, Z., Pace, U., Heldman, J., and Lancel, D. (1986) J. Neurosci. 6, 2146–2154
27. Danner, S., and Lohse, M. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10145–10150
28. Pershadsingh, H. A., and McDonald, J. M. (1980) J. Biol. Chem. 255, 4087–4093
29. Boekhoff, I., Tareilus, E., Strotmann, J., and Breer, H. (1990) EMBO J. 9, 2453–2458
30. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
31. Xu, J., Wu, D., Slepak, V. Z., and Simon, M. (1995) Proc. Natl. Acad. Sci. 92, 2096–2099
32. Winstel, R., Freund, S., Krasel, C., Hoppe, E., and Lohse, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2105–2109