The α2-adrenergic receptor (α2AR) subtype α2C10 undergoes rapid agonist-promoted desensitization which is due to phosphorylation of the receptor. One kinase that has been shown to phosphorylate α2C10 is an agonist-dependent manner is the βARK kinase (βARK), a member of the family of G protein-coupled receptor kinases (GRKs). In contrast, the α2C4 subtype has not been observed to undergo agonist-promoted desensitization or phosphorylation by βARK. However, the substrate specificities of the GRKs for phosphorylating α2AR subtypes are not known. We considered that differential capacities of various GRKs to phosphorylate α2C10 and α2C4 might be a key factor in dictating in a given cell the presence or extent of agonist-promoted desensitization of these receptors. COS-7 cells were co-transfected with α2C10 or α2C4 without or with the following GRKs: βARK, βARK2, GRK5, or GRK6. Intact cell phosphorylation and receptor phosphorylation (7). Taken together, these results implicate phosphorylation of α2C10 by βARK as one of the key components in short term agonist-promoted desensitization. However, βARK is only one of several members of a family of kinases, termed GRKs, which phosphorylate the agonist-occupied form of G protein-coupled receptors (8). Other members of this kinase family that have been cloned and found to have diverse tissue distributions include βARK2 (GRK3), GRK5, and GRK6 (9–11). It is not known whether these other kinases phosphorylate α2C10 in a manner similar to that of βARK (GRK2). In regard to α2ARs, another subtype, the human α2C4AR (α2C4), fails to undergo agonist-promoted phosphorylation or functional desensitization in model systems examined thus far (3, 6). This is intriguing, because the third intracellular loop of this receptor has a number of serines or threonines that may be potential GRK phosphorylation sites. It is not known whether α2C4 could be a substrate for some of these other GRKs, such that the lack of desensitization/phosphorylation reported to date may be due to lack of expression of such kinases in the cell systems utilized.

We therefore undertook the current studies to delineate whether α2C10 and α2C4 phosphorylation and desensitization...
Substrate Specificity of GRKs for \( \alpha_2 \)ARs

is GRK isomeric-specific. The studies were designed to answer two questions: 1) which GRKs phosphorylate \( \alpha_2 \)C10 and 2) do any of the known GRK's phosphorylate \( \alpha_2 \)C4? To approach this, we co-expressed each receptor subtype individually with each of the four kinases and assessed agonist-promoted phosphorylation of the receptor in the intact cell setting. To confirm the significance of such phosphorylation (or lack thereof), we also developed a transient expression system whereby functional desensitization of each of the two \( \alpha_2 \)AR subtypes was determined in cells with or without co-transfection with a given GRK. The results indicate that agonist-promoted \( \alpha_2 \)AR desensitization is both receptor subtype and GRK isoform selective.

EXPERIMENTAL PROCEDURES

Tissue Culture and Transfections—COS-7 cells were maintained in Dulbecco's modified Eagle's medium and HEK293 cells were maintained in Earl's minimal essential medium, each supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 \( \mu \)g/ml streptomycin at 37 °C, 5% CO2. The DEAE-dextran method of transfection was used to introduce plasmid DNA into each cell type for transient expression. The common expression vector utilized for expression of the \( \alpha_2 \)ARs and GRKs was pBC121B as reported previously (7). Therefore, for COS-7 cells, 10 \( \mu \)g of pBC-C10 or pBC-C4 and 10 \( \mu \)g of pBC121B containing the large T antigen, they do not transiently express cDNAs derived from HEK293 cells was developed. HEK293 cells were transiently transfected such that either \( \alpha_2 \)C4 or \( \alpha_2 \)C10 was expressed along with the \( \alpha_2 \)A dopamine receptor and a given GRK. Thus, \( \alpha_2 \)AR-mediated inhibition of dopamine-stimulated cAMP accumulation was utilized as the assay for assessment of GRK function. Using such an approach, dopamine (0.5 \( \mu \)M) stimulated transfected HEK293 cell cAMP levels ~5-fold over basal levels. Co-incubation with the \( \alpha_2 \)AR agonist UK14304 inhibited dopamine-stimulated cAMP accumulation by ~70% in a dose-dependent manner. (In nontransfected cells, neither dopamine or UK14304 had any effect on cAMP accumulation). For agonist-promoted desensitization studies, cells were exposed to the same conditions as those of the phosphorylation studies (see above) except that the hydrophilic \( \alpha_2 \)AR agonist PGE-6201204 was used. After washing five times in cold PBS, cells were gently tapped to dislodge them from the flask, centrifuged at 400 \( \times \) g at 4 °C, and resuspended in Earle's minimal essential medium containing 100 \( \mu \)M ascorbic acid. Cells were then incubated for 5 min in a 200-\( \mu \)l volume of medium containing 0.5 \( \mu \)M dopamine, 100 \( \mu \)M ascorbic acid, and eight concentrations (0–1 mM) of UK14304. Reactions were terminated by the addition of 20 \( \mu \)l of 1 M HCl. Cellular cAMP production was then measured using a radioimmunoassay as described previously (16).

Materials—Tissue culture supplies were purchased from JRH Biologicals, and radiolabels were from DuPont NEN. The cDNAs and the antisera for the various GRKs were kindly provided by Dr. J. Benovic, Thomas Jefferson University, Jefferson Medical College, and the antisera directed against GRK6, which also recognizes GRK5, for desensitization purposes, autoradiograms were produced by exposing the gels to X-ray film for 16 h.

Functional Desensitization and cAMP Determination—In order to delineate the relevance of the results from receptor phosphorylation experiments, a system to assess receptor function and desensitization in HEK293 cells was developed. HEK293 cells were transiently transfected such that either \( \alpha_2 \)C4 or \( \alpha_2 \)C10 was expressed along with the \( \alpha_2 \)A dopamine receptor and a given GRK. Thus, \( \alpha_2 \)AR-mediated inhibition of dopamine-stimulated cAMP accumulation was utilized as the assay for assessment of GRK function. Using such an approach, dopamine (0.5 \( \mu \)M) stimulated transfected HEK293 cell cAMP levels ~5-fold over basal levels. Co-incubation with the \( \alpha_2 \)AR agonist UK14304 inhibited dopamine-stimulated cAMP accumulation by ~70% in a dose-dependent manner. (In nontransfected cells, neither dopamine or UK14304 had any effect on cAMP accumulation). For agonist-promoted desensitization studies, cells were exposed to the same conditions as those of the phosphorylation studies (see above) except that the hydrophilic \( \alpha_2 \)AR agonist PGE-6201204 was used. After washing five times in cold PBS, cells were gently tapped to dislodge them from the flask, centrifuged at 400 \( \times \) g at 4 °C, and resuspended in Earle's minimal essential medium containing 100 \( \mu \)M ascorbic acid. Cells were then incubated for 5 min in a 200-\( \mu \)l volume of medium containing 0.5 \( \mu \)M dopamine, 100 \( \mu \)M ascorbic acid, and eight concentrations (0–1 mM) of UK14304. Reactions were terminated by the addition of 20 \( \mu \)l of 1 M HCl. Cellular cAMP production was then measured using a radioimmunoassay as described previously (16).

RESULTS

To delineate which GRKs phosphorylate the \( \alpha_2 \)C10 and \( \alpha_2 \)C4 receptors, these receptors were transiently expressed in COS-7 cells along with \( \beta \)ARK, \( \alpha_2 \)ARK2, GRK5, or GRK6. Expression of the receptors as assessed by \( ^{3} \)H)hoyhimbine, with phenolamine to determine nonspecific binding, as described previously (3). Dose-response curves for cAMP inhibition were fit by a nonlinear iterative least squares technique using software from GraphPad (San Diego, CA).

Materials—Tissue culture supplies were purchased from J RH Biosciences and radiolabels were from DuPont NEN. The cDNAs and the antisera for the various GRKs were kindly provided by Dr. J. Benovic, Thomas Jefferson University, Jefferson Medical College, and the pRSV-T antigen vector was obtained from Dr. R. Reed, The Johns Hopkins University School of Medicine. UK14304 was from Research Biochemicals International (Natick, MA) and PGE-6201204 was from Procter and Gamble Pharmaceuticals (Cincinnati, OH). All other reagents were purchased from sources listed previously (2, 7, 15).

RESULTS

To delineate which GRKs phosphorylate the \( \alpha_2 \)C10 and \( \alpha_2 \)C4 receptors, these receptors were transiently expressed in COS-7 cells along with \( \beta \)ARK, \( \alpha_2 \)ARK2, GRK5, or GRK6. Expression of the receptors as assessed by \( ^{3} \)H)hoyhimbine binding was typically ~5 pmol/mg. Expression of the GRKs was assessed by Western blots (Fig. 1). As is shown, using the methods outlined and the antisera directed against \( \beta \)ARK (which also recognizes \( \alpha_2 \)ARK2), \( \beta \)ARK was found to be expressed in nontransfected COS-7 cells. Upon transfection with pBC-\( \beta \)ARK, expression was increased >15-fold. Under these conditions, \( \beta \)ARK2 was not detected in nontransfected cells. However, \( \beta \)ARK2 was clearly expressed after transfection with pBC-\( \beta \)ARK2. The results of Western blots for GRK5 and GRK6, using antisera directed against GRK6, which also recognizes GRK5, are shown in Fig. 1B. A weak band, which could represent either kinase, was noted in nontransfected cells. Upon transfection with the GRK5 or GRK6 constructs, expression of the respective kinases was markedly enhanced. Western blots of \( \alpha_2 \)C10 and \( \alpha_2 \)C4 in membranes isolated from COS-7 cells following
treatment in either medium alone or medium plus agonist for 20 min were performed in order to confirm that the receptors are recognized by the antibodies under both conditions. As seen in Fig. 2, α2C10 and α2C4 are recognized by their respective antibodies in the agonist occupied and nonoccupied forms of the receptors to the same extent.

To determine whether enhanced expression of the various kinases augments phosphorylation of α2C10 and α2C4, whole cell phosphorylation studies were carried out using COS-7 cells separately expressing the two receptor subtypes and overexpressing each GRK. Fig. 3A shows an autoradiograph of a representative experiment with α2C10, and Fig. 3B summarizes the results from multiple experiments. It is clear that there is detectable agonist-promoted phosphorylation of α2C10 in COS-7 cells not transfected with any GRK. Cells overexpressing βARK or βARK2, however, showed higher levels of receptor phosphorylation (220 ± 30% for βARK and 210 ± 30% for βARK2 co-transfections, as compared with non-GRK-transfected, p < 0.01). In contrast, levels of α2C10 phosphorylation did not change upon overexpression of GRK5 or GRK6 (i.e., 90 ± 30% for GRK5 and 80 ± 50% for GRK6, p = not significant). Identical experiments were performed to determine whether α2C4 is phosphorylated by agonist under conditions of overexpression of these kinases. Fig. 4A shows a representative experiment. In the absence of overexpression of a GRK, α2C4 does not appear to undergo any agonist-mediated phosphorylation. Nor is this receptor phosphorylated under any of the other circumstances tested (overexpression of βARK, βARK2, GRK5, or GRK6). α2C10 co-expressed with βARK was used as a positive control for these studies. We have interpreted the above experiments as being consistent with α2C10 being a substrate for βARK and βARK2, but not GRK5 or GRK6. For α2C4, none of the kinases appear to phosphorylate the receptor under the conditions studied.

From the results of the above phosphorylation experiments, we would predict that overexpression of certain kinases (such as βARK) would augment agonist-promoted desensitization of α2C10, while such overexpression of other kinases (such as GRK6) would not. Furthermore, for α2C4, which does not undergo agonist-promoted phosphorylation, we would not expect to see a gain of desensitization with overexpression of any GRK. While COS-7 cells provide for the high levels of expression of receptors and kinases required for the phosphorylation studies, functional studies are problematic, since transfected α2AR expressed in COS-7 cells mediate a complex pattern of cAMP modulation due to dual coupling to Gs and Gi (18–20). A model system was thus developed in HEK293 cells. With transient expression of α2AR in these cells, agonist-mediated inhibition of cAMP is easily observed. With overexpression of
played no agonist-promoted desensitization as indicated by no shift in the dose-response curve or change in the maximal degree of inhibition. These results are in agreement with what was found with the phosphorylation studies, in that agonist-promoted phosphorylation of \( \alpha_2 \)C10 was augmented by \( \beta \)ARK but not GRK6. For \( \alpha_2 \)C4, functional desensitization was not observed when \( \beta \)ARK was overexpressed (Table I), also consistent with the phosphorylation studies.

Several mechanisms responsible for agonist-promoted desensitization of \( \alpha_2 \)AR have been established (reviewed in Refs. 1 and 21). Four different processes have thus far been identified: phosphorylation, sequestration, down-regulation, and decreases in cellular \( G_i \). For those \( \alpha_2 \)AR subtypes that do display rapid agonist-promoted desensitization, the predominant mechanism responsible for such short term regulation is phosphorylation of the receptor (2, 6, 22). Down-regulation of receptor expression and changes in cellular \( G_i \) are consequences of prolonged agonist exposure, and sequestration probably serves to dephosphorylate the receptor, but has not been clearly implicated in rapid desensitization.

That \( \beta \)ARK, or a related GRK, is the kinase that phosphorylates \( \alpha_2 \)C10 and mediates agonist-promoted desensitization was first suggested by in vitro studies with purified platelet \( \alpha_2 \)AR and purified bovine \( \beta \)ARK. In a reconstituted phospholipid vesicle system, \( \alpha_2 \)AAR was shown to undergo phosphorylation by \( \beta \)ARK in an agonist-dependent fashion (4). However, the relevance of this finding to receptor regulation in intact cells was not clear, and indeed even the presence of agonist-promoted desensitization of \( \alpha_2 \)AR was debated. Subsequent studies using recombinant \( \alpha_2 \)C10 expressed in Chinese hamster fibroblasts (CHO cells) and CHW cells have clearly established that this receptor undergoes agonist-promoted desensitization (2, 3, 6). Such desensitization was found to occur rapidly (within minutes of agonist exposure) and correlated with receptor phosphorylation as assessed in intact cell studies. \( \alpha_2 \)C10 desensitization in CHW cells is blocked by heparin, a known inhibitor of \( \beta \)ARK phosphorylation, but not by inhibitors of protein kinases A or C (2). In vitro phosphorylation studies using synthetic peptides and purified \( \beta \)ARK have shown that a peptide with sequence identical to that of a \( \beta \)ARK substrate for \( \alpha_2 \)AR has also been established (reviewed in Refs. 18085). In vitro phosphorylation and agonist-promoted desensitization of \( \alpha_2 \)AR has also been found in CHO cells recombinantly expressing the \( \alpha_2 \)AR subtypes. The \( \alpha_2 \)C10 was found to display agonist-promoted phosphorylation and functional desensitization, while \( \alpha_2 \)C4 did not phosphorylate or desensitize (3, 6). In addition, in COS-7 cells it has been reported that co-expression of \( \beta \)ARK enhances

---

**Fig. 4.** Agonist-promoted phosphorylation of \( \alpha_2 \)C4 co-expressed with and without the various GRKs. Whole cell phosphorylation experiments were performed using COS-7 cells expressing \( \alpha_2 \)C4 and overexpressing each GRK as in Fig. 3. As a control for phosphorylation, \( \alpha_2 \)C10 + \( \beta \)ARK was included in these experiments. A shows a representative experiment, while B summarizes the results of three independent experiments. In the absence of overexpression of a GRK, \( \alpha_2 \)C4 does not appear to undergo any agonist-mediated phosphorylation. Likewise, this receptor is not phosphorylated under any of the conditions tested (i.e. upon overexpression of \( \beta \)ARK, \( \beta \)ARK2, GRK5, or GRK6).

\( \alpha_2 \)C10, the endogenous kinases were insufficient to provide for detectable agonist-promoted desensitization as indicated by no shift in the dose-response curve or change in the maximal degree of inhibition. This then allowed us to assess the effect of a given kinase on \( \alpha_2 \)AR desensitization by also overexpressing the kinase. For these studies, two kinases, which gave different results in the phosphorylation experiments, \( \beta \)ARK and GRK6, were utilized. In all experiments, the D1A dopamine receptor was also expressed to provide for a receptor for stimulation of adenylyl cyclase. As shown in Fig. 5, transfection of the \( \beta \)ARK and GRK6 constructs indeed resulted in an increase in expression of each kinase as determined by Western blots. Under the above conditions, agonist exposure for 20 min had no effect on \( \alpha_2 \)C10 function in the absence of GRK overexpression (Table I). Thus, no change in the EC\(_{50}\) for UK14304-mediated inhibition of CAMP accumulation, nor in the maximal extent of inhibition, was noted (Table I). However, when \( \beta \)ARK was overexpressed, a clear desensitization of \( \alpha_2 \)C10 was observed. In these cells, the EC\(_{50}\) increased ~6.4-fold after agonist exposure with a ~17% decrease in the maximal degree of inhibition (Table I). (It should be noted that the mean basal EC\(_{50}\) for inhibition with \( \beta \)ARK co-expression trended toward being slightly lower than without \( \beta \)ARK, but this was not consistent and was not statistically significant). When examined at a submaximal concentration of UK14304 in the assay (10 \( \mu \)M), the observed shift in the dose response curve was found to result in ~50% desensitization of the receptor (from ~47 to ~25% inhibition). In contrast, cells overexpressing GRK6 displayed no agonist-promoted desensitization as indicated by no

---

**Fig. 5.** Western analysis of \( \beta \)ARK and GRK6 expressed in HEK293 cells. To confirm overexpression of \( \beta \)ARK and GRK6 in HEK293 cells after transfection, a Western analysis was performed using protein isolated from whole cells as described under “Experimental Procedures.”
agonist-promoted phosphorylation of recombinantly expressed α2C10 (6). Recently, studies utilizing site-directed mutagenesis of the predicted βARK phosphorylation sites within the sequence EESSSS of the third intracellular loop of α2C10 have been reported (7). In these studies, substitutions of selected serines with alanines were carried out and intact cell phosphorylation and desensitization in CHO cells assessed. The results indicated that all four serines (residues 296–299) are phosphorylated by βARK during agonist exposure and that such phosphorylation is obligatory for functional desensitization. Taken together, these studies strongly indicate phosphorylation by βARK as a key mediator of short-term agonist-promoted desensitization of α2C10 (also further reviewed in Refs. 1 and 21).

Whether α2C10 or α2C4 serve as substrates for other members of the GRK family has not been investigated. Regarding α2C10, we wondered whether other GRKs have the potential to phosphorylate this receptor, while with α2C4 we were interested in whether any of these kinases could subsist this function. We considered that such kinase specificity might provide for cell-type differences in the desensitization response of α2AR. Based on the diversity of primary structure of the known GRKs, differences in their requirement for substrate recognition/phosphorylation is clearly plausible (8). However, to date little is known about substrate specificities of the different kinases using intact receptors expressed in cells. To address this with the α2ARs, we studied α2C10, which has been shown to undergo desensitization, and α2C4, which does not appear to undergo desensitization in the model systems examined thus far. Whole cell intact receptor phosphorylation studies were carried out in COS-7 cells co-expressing α2C10 or α2C4 with βARK, βARK2, GRK5, or GRK6 in the absence or presence of agonist. Both βARK and βARK2 were found to phosphorylate α2C10 in an agonist-dependent manner, while GRK5 and GRK6 did not. α2C4 was not phosphorylated by any of the kinases studied. To assess whether such phosphorylation (or lack thereof) had a physiologic correlate, functional studies were carried out in cells with or without co-expression of specific kinases. Consistent with what was found in the phosphorylation studies, α2C10 desensitization was imparted by co-expression of βARK, while there was no effect upon co-expression of GRK6. For α2C4, βARK overexpression did not induce desensitization. We conclude from these studies that α2AR desensitization displays both receptor subtype and GRK isoform specificity. We and others had previously shown in CHO cells or COS-7 cells that α2C10 underwent agonist-promoted phosphorylation and desensitization, but that α2C4 does not (3, 6). Based on the current study, it appears that this lack of desensitization is not due to a lack of expression of a particular kinase in these cells. Rather, it appears that α2C4 is not a substrate for any of the kinases tested. For α2C10, which was known to be phosphorylated by βARK, it was not clear which of the other GRKs could also subsist this function. Based on the current study, α2C10 appears to be a substrate for βARK and βARK2, but not GRK5 or GRK6.

GRK substrate specificity is consistent with a number of key differences among the mammalian kinases dosed to date. For example, the kinases appear to possess distinct mechanisms responsible for their localization to the membrane. βARK and βARK2 contain binding sites within their C terminus for the βγ subunits of heterotrimeric G proteins (23, 24), while GRK5 appears to be constitutively associated with the membrane possibly due to a stretch of basic amino acids localized in its C terminus, which interact with phospholipid head groups (25). The mechanism for membrane localization of GRK6 is distinct from the others in that it appears to involve palmitoylation (26). The substrate specificity of the various kinases, as determined by peptide studies, has also been shown to differ. While βARK and βARK2 preferentially phosphorylate serine or threonine residues C-terminal to acidic amino acids (5), GRK5 and GRK6 do not appear to display such a preference. Another difference among the kinases involves their regulation by autophosphorylation (27, 28). While GRK5 is autophosphorylated (29), likely leading to its activation, βARK, βARK2, and GRK6 are not (28).

That one of the α2AR subtypes does not undergo agonist-promoted phosphorylation by any of these GRKs furthers the notion that this property may be a distinguishing feature between structurally related receptor subtypes. α2C10 and α2C4 have similar affinities for endogenous catecholamines and both couple to Gi and, though less efficiently, to Gs as well. Yet α2C4 lacks the molecular requirements for GRK phosphorylation, which has turned out to be the most obvious difference between the two receptors reported to date. Similarly, within the βAR subtypes, the β2AR does not undergo rapid agonist-promoted phosphorylation/desensitization (30), which is in contrast to β1 and β3AR. Thus, receptors such as α2C4 and β2AR may have evolved to be resistant to this adaptive response. That only some of the GRKs phosphorylate α2C10 provides for yet another level of complexity. Based on the current findings it can be predicted that α2C10 on cells expressing exclusively a GRK5/GRK6-type kinase would not be expected to undergo rapid desensitization while in another cell which expressed a βARK/βARK2-type kinase, α2C10 desensitization would occur. As we learn more about the localization of the different GRKs at the cellular level within various organs, these concepts can be assessed in regard to specific physiologic responses.

Acknowledgments—We express our appreciation to Dr. J. Benovic for providing the antisera and the cDNAs for the various GRKs and Dr. R.
Reed for providing the pRSV-T vector. We also thank Cheryl Theiss and Elizabeth Donnelly for technical assistance and Katie Gouge for manuscript preparation.

Note Added in Proof—Recently, Cottecchia and colleagues have reported receptor subtype and GRK specificity for agonist-promoted desensitization of the α2-adrenergic receptor (31).

REFERENCES
1. Liggett, S. B., and Lefkowitz, R. J. (1993) in Regulation of Cellular Signal Transduction Pathways by Desensitization and Amplification (Sibley, D., and Houslay, M., eds) pp. 71–97, John Wiley & Sons Ltd., London
2. Liggett, S. B., Ostrowski, J., Chestnut, L. C., Kurose, H., Raymond, J. R., Caron, M. G., and Lefkowitz, R. J. (1992) J. Biol. Chem. 267, 4740–4746
3. Eason, M. G., and Liggett, S. B. (1992) J. Biol. Chem. 267, 25473–25479
4. Benovic, J. L., Regan, J. W., Matsu, H., Mayor, F. Jr., Cottecchia, S., Lee-Lundberg, L., Caron, M. G., and Lefkowitz, R. J. (1987) J. Biol. Chem. 262, 17251–17253
5. Onorato, J. J., Palczewski, K., Regan, J. W., Caron, M. G., Lefkowitz, R. J., and Benovic, J. L. (1991) Biochemistry 30, 5118–5125
6. Kurose, H., and Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 10993–10099
7. Eason, M. G., Moreira, S. P., and Liggett, S. B. (1995) J. Biol. Chem. 270, 4681–4688
8. Lefkowitz, R. J. (1993) Cell 74, 409–412
9. Benovic, J. L., Onorato, J. J., Arriza, J. L., Stone, W. C., Loise, M., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., Caron, M. G., and Lefkowitz, R. J. (1991) J. Biol. Chem. 266, 14935–14946
10. Kurose, H., and Lefkowitz, R. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 90, 5388–5392
11. Benovic, J. L., and Gomez, J. (1993) J. Biol. Chem. 268, 19521–19527
12. De chasseval, R., and de Villartay, J. P. (1995) Nucleic Acids Res. 20, 245–250
13. Luttrell, L. M., Ostrowski, J., Cottecchia, S., Kendall, H., and Lefkowitz, R. J. (1993) Science 259, 1453–1457
14. Kurose, H., Arriza, J. L., and Lefkowitz, R. J. (1993) Mol. Pharmacol. 43, 444–450
15. Jewell-Motz, E. A., and Liggett, S. B. (1995) Biochemistry 34, 11946–11953
16. Green, S., and Liggett, S. B. (1994) J. Biol. Chem. 269, 26215–26219
17. Smith, P. K., Krohn, R. I., Hermanson, G. T., Maila, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) Anal. Biochem. 150, 76–85
18. Federman, A. D., Conklin, B. R., Schrader, K. A., Reed, R. R., and Bourne, H. R. (1992) Nature 356, 159–161
19. Eason, M. G., Jacinto, M. T., and Liggett, S. B. (1994) Mol. Pharmacol. 45, 696–702
20. Eason, M. G., and Liggett, S. B. (1995) J. Biol. Chem. 270, 24753–24760
21. Liggett, S. B. (1996) in Alpha2-adrenergic Receptors: Structure, Function and Therapeutic Implications (Lanier, S., and Limbird, L., eds) Harwood Academic Publishers GmbH, Reading, UK
22. Eason, M. G., and Liggett, S. B. (1992) J. Biol. Chem. 267, 25473–25479
23. Pitcher, J. A., Inglese, 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. (1995) Science 275, 1264–1267
24. Koch, W. J., Inglese, J., Stone, W. C., and Lefkowitz, R. J. (1993) J. Biol. Chem. 268, 8256–8260
25. Premont, R. T., Koch, W. J., Inglese, J., and Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 6832–6841
26. Stoffel, R. H., Randall, R. R., Premont, R. T., Lefkowitz, R. J., and Inglese, J. (1994) J. Biol. Chem. 269, 27791–27794
27. Kunapuli, P., Onorato, J. J., Husey, M. M., and Benovic, J. L. (1994) J. Biol. Chem. 269, 1099–1105
28. Loudon, R. P., and Benovic, J. L. (1995) J. Biol. Chem. 269, 22691–22697
29. Kunapuli, P., Gurevich, V. V., and Benovic, J. L. (1995) J. Biol. Chem. 269, 10209–10212
30. Liggett, S. B., Freedman, N. J., Schwinn, D. A., and Lefkowitz, R. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3665–3669
31. Diviani, D., Lattion, A. L., Larbi, N., Kunapuli, P., Pronin, A., Benovic, J. L., and Cottecchia, S. (1996) J. Biol. Chem. 271, 5049–5058