Expression of Phosducin in a Phosducin-negative Cell Line Reveals Functions of a G\(_{\beta\gamma}\)-binding Protein*

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Phosducin is a member of the large group of proteins that bind to G-protein \(\beta\gamma\)-subunits (G\(_{\beta\gamma}\)) and whose biological functions are often unknown. Human A431 cells do not contain detectable amounts of phosducin. We generated A431 cells expressing phosducin at a level of \(\sim 1\) pmol/mg of cytosolic protein, which is \(\sim 10\%\) of the phosducin level in brain. cAMP accumulation in response to \(\beta\)-adrenergic receptor agonists was enhanced at early times in phosducin-expressing cells, but reached a lower plateau than in control cells. Permeabilization of the cells with digitonin did not change this pattern, but allowed the introduction of specific inhibitors: antibodies to phosducin abolished all differences between the two cell lines. Inhibitors of the \(\beta\)-adrenergic receptor kinase abolished all differences at early time points. An almost complete loss of \(\beta\)-adrenergic receptor desensitization in the phosducin-expressing cells was also observed when intact cells were desensitized and receptor function was then determined in membrane preparations. Inhibition of protein kinase \(A\) accentuated the effects of phosducin, suggesting that also in \textit{vivo} phosducin is regulated by this kinase. These data indicate that phosducin affects G-protein-mediated signaling in at least two ways: it dampens the overall responsiveness, and it impairs the rapid desensitization mediated by the \(\beta\)-adrenergic receptor kinase.

A large array of membrane receptors utilize GTP-binding G-proteins to transmit their signals across membranes. These G-proteins are heterotrimeric proteins consisting of \(\alpha\), \(\beta\), and \(\gamma\)-subunits (1–3). They couple activated seven-transmembrane helix receptors to divergent effectors such as adenylyl cyclase and other enzymes or to various ion channels. These signaling pathways are highly regulated systems. Such regulation has been described mostly at the receptor level, where multiple mechanisms are capable of reducing receptor function at various speeds and for various periods of time (4, 5).

Classically, most functions of G-proteins have been assigned to the \(\alpha\)-subunits. However, over the past years it has become clear that the \(\beta\gamma\)-subunits (G\(_{\beta\gamma}\)) have multiple signaling functions of their own (6–8). G\(_{\beta\gamma}\) have been shown to interact with a growing number of divergent proteins, and these G\(_{\beta\gamma}\)-binding proteins have recently gained much interest both from a structural and functional point of view. From a structural point, several of the G\(_{\beta\gamma}\)-binding proteins appear to contain a pleckstrin-homology (PH) domain (9), a domain characterized by a six-membered \(\beta\)-barrel closed by a terminal \(\alpha\)-helix (10, 11). In two cases of PH domain-containing proteins, the \(\beta\)-adrenergic receptor kinase, and Ras guanine nucleotide releasing factor, the G\(_{\beta\gamma}\)-binding region has been mapped to the C-terminal segment of the domain, encompassing the \(\alpha\)-helix and extending beyond it (12, 13). A short consensus sequence Gln/Asn-X-Glu/Asp-Arg/Lys has been proposed as the essential determinant of G\(_{\beta\gamma}\) coupling (14). On the functional level it has been shown that G\(_{\beta\gamma}\) binding proteins can inhibit G\(_{\beta\gamma}\)-mediated signaling in reconstituted systems, in overexpressing cells and in transgenic mice (15–17). These experiments underline the importance of G\(_{\beta\gamma}\)-mediated signaling. However, they do not elucidate the physiological roles of G\(_{\beta\gamma}\)-binding proteins, since the overexpression resulted in non-physiological levels, and since the overexpressed protein was truncated. A more relevant strategy to elucidate their function would be the knock-out of a G\(_{\beta\gamma}\)-binding protein. However, a much simpler approach is the re-introduction of such a protein, at physiological levels, into a background where it is not present.

We have chosen phosducin as a G\(_{\beta\gamma}\)-binding protein. Phosducin was initially discovered as a major retinal phosphoprotein which could be copurified with the \(\beta\) and \(\gamma\)-subunits of G\(_t\), the retinal G-protein (18). Its expression had been thought to be restricted to the retina and the developmentally related pineal gland (19). Phosducin was subsequently purified from bovine brain and its mRNA was identified in many tissues, suggesting that it is widely distributed (20). Recombinant purified phosducin has been shown to inhibit the GTPase activity of several purified G-proteins; furthermore, addition of phosducin to cell membranes inhibited the stimulation of adenylyl cyclase by \(\beta\)-adrenergic receptors or by G\(_t\). From these data we concluded that phosducin might be a widely distributed G-protein regulator (20). Similar inhibitory effects were also observed for G\(_t\) (21).

In contrast to most other G\(_{\beta\gamma}\)-binding proteins, phosducin has no other known enzymatic or signaling function, and may thus be regarded as a “pure” G\(_{\beta\gamma}\)-binding protein. Furthermore, it has a high affinity for G\(_{\beta\gamma}\), suggesting that G\(_{\beta\gamma}\)-binding might be its primary physiological role. We wished to explore the functions that phosducin might have when present at physiological levels. For these studies, we took advantage of the fact that we could not detect phosducin in human A431 cells, a cell line widely used to characterize \(\beta\)-adrenergic receptor/G\(_{\beta\gamma}\)-mediated signaling, and generated cell lines stably expressing phosducin at physiological levels.

**EXPERIMENTAL PROCEDURES**

**Vector Construction**—A 738-base pair NcoI-BamHI fragment containing the coding region for bovine phosducin was excised from the vector pET-phd (20), blunted with the Klaven polymerase fragment, and ligated into the blunted ApaI and SmaI sites of the expression vector pBC-KS-dhfr (22). The resultant vector, termed pBC-phd-dhfr, contains the phosducin cDNA under the control of the strong cytomeg-
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alovirus immediate early promoter, and the mouse dihydrofolate reductase promoter to generate a bovine phosducin gene expression vector with the control block of the SV40 promoter (above curve, 1:200 dilution), the βARK inhibitors heparin (100 nm) and the peptide KTIAKFERLQTVTNYFITSE (50 μM; Ref. 27), or the PKA inhibitor peptide PKI (10 μM) were added immediately after the permeabilization. 10 min later (−)-isoproterenol was added and cAMP accumulation was measured as described above for intact cells.

β-Adrenergic Receptor Desensitization in Intact A431 Cells—Desensitization of β2-adrenergic receptors in intact A431 cells was effected by incubating the cells for 10 min at 37 °C in Dulbecco’s modified Eagle’s medium containing 10 μM (−)-isoproterenol. The incubation was stopped by washing the cells three times in ice-cold phosphate-buffered saline. Subsequently, crude membranes were prepared and the extent of desensitization was determined in adenylyl cyclase assays as described below. The adenylyl cyclase assay used to screen for adenylate cyclase activation were done with millimolar concentrations of Mg2+, conditions that favor the detection of βARK-mediated versus PKA-mediated desensitization (26, 28).

Adenylyl Cyclase Assays in A431 Cell Membranes—Cells were harvested in 50 mM Tris-HCl, pH 7.4, and disrupted with an Ultra-Turrax homogenizer. Crude membranes were prepared by centrifugation of the homogenate at 50,000 × g for 30 min, followed by resuspension in 50 mM Tris-HCl, pH 7.4, and a similar centrifugation step. The final pellet was resuspended in 1 ml of 50 mM Tris-HCl, pH 7.4. Adenylyl cyclase activity in the crude membranes was determined essentially as described (29). Incubations contained up to 50 μM of crude membrane protein, 50 mM Tris-HCl, pH 7.4, 4 mM MgCl2, 1 mM EDTA, 100 μM [α-32P]ATP (0.2 mM/Cu tube). An aliquot of 100 μg (0.1 mg/ml) of prewashed protein A-Sepharose (Pharmacia) for 30 min at 4 °C. After centrifugation, 5 μg of affinity purified rabbit anti-phosducin antibodies were added to the supernatant and incubated for 1 h. The antibodies were bound to another portion of protein A-Sepharose and pelleted by centrifugation. After washing five times in 50 mM Tris-HCl, pH 7.4, containing 1% Brij 96 (Sigma), 50 mM NaCl, 10 mM EDTA, 20 mg/liter benzamidine, and 20 μM phenylmethylsulfonyl fluoride, the samples were resuspended in SDS sample buffer, heated to 95 °C for 5 min to release bound protein, and subjected to Western analysis.

For Western blots, samples were harvested in phosphate-buffered saline and disrupted by sonicating. Cytosolic and particulate fractions were separated by centrifugation at 200,000 × g for 30 min. Samples containing 200 μg of protein per lane were separated by electrophoresis on 12% SDS-polyacrylamide gels and blotted onto Immobilon (Millipore) membranes. Goat antibodies (1:2,000 dilution) and horseradish peroxidase-coupled secondary antibodies (Dianova) were used in conjunction with ECL reagents (Amersham) to develop the blots.

RESULTS

In order to find a phosducin-negative cell line suitable for expression of phosducin and for the analysis of its effects on G-protein-mediated signaling, we tested a variety of cell lines for the presence of phosducin in Western blots with and without prior immunoprecipitation. Using these techniques, which had high sensitivity well beyond the level of detection, we could not detect any phosducin in human A431 cells (see Fig. 1), a cell line with a well characterized β-adrenergic receptor/G signaling system. A431 cells stably expressing phosducin were then

1 The abbreviations used are: IBMX, 3-isobutyl-1-methylxanthine; βARK, β-adrenergic receptor kinase; PKA, protein kinase A; PKI, protein kinase A inhibitor peptide.
generated by transfection with the expression vector pBC-phd-dhfr followed by selection with genetin and methotrexate. A control cell line was generated by similar transfection and selection using the control vector pBC-KS-dhfr. Fig. 1A shows the expression of phosducin in the two cell lines as visualized in a Western blot. Using purified recombinant phosducin as a standard, the extent of expression was estimated at ~1 pmol of phosducin/mg of cytosolic protein. Subcellular fractionation studies showed that almost all phosducin was present in the cytosol, whereas only very weak phosducin immunoreactivity was found in particulate fractions (data not shown). In the control cell line, phosducin could not be detected with our antibodies in Western blots when up to 200 μg of cellular protein were loaded per lane (Fig. 1); since less than 10 fmol of phosducin could be detected in such blots, this suggests a level of <50 fmol/mg protein in the control cells.

The level of phosducin expression was then compared with the endogenous level found in brain. This was done using immunoprecipitation followed by Western blots with another antibody, because in experiments with brain tissue we could not obtain clear bands in direct Western blots. The efficiency of this immunoprecipitation is ~20%. Much more phosducin immunoreactivity was found in brain than in the phosducin-expressing A431 cells, whereas there was no signal from control A431 cells (Fig. 1B). A semiquantitative analysis using recombinant phosducin as a standard (not shown) gave phosducin levels of ~10 pmol/mg of cytosolic protein in bovine brain. Thus, the level of phosducin expression in the A431 cells was well within the physiological range.

The expression of G\(_\beta\) (quantitated in Western blots) and G\(_\beta\) subunits (determined by radioligand binding) was not affected by phosducin expression (Table I). Likewise, total βARK activity measured in cytosolic preparations with light-activated rhodopsin as the substrate was identical in control and in phosducin-expressing cells (Table I).

In order to investigate the effects of phosducin on G-protein-mediated signaling, we measured the cAMP accumulation caused by stimulation of β\(_2\)-adrenergic receptors (Fig. 2). These experiments were done in the presence of the phosphodiesterase inhibitor IBMX to inhibit cAMP degradation. The β\(_2\)-adrenergic receptor agonist isoproterenol at 10 μM caused a more than 100-fold increase of the cellular cAMP content. This increase was clearly reduced in phosducin-expressing cells. At steady-state, the cAMP levels were almost 3 fmol/control cell compared to just above 1 fmol/phosducin-expressing cell. This indicates that phosducin exerts an inhibitory effect on the β\(_2\)-adrenergic receptor/G\(_\alpha\)/adenylyl cyclase system.

However, the kinetics of isoproterenol-induced cAMP accumulation were more rapid in the phosducin-expressing cells. The time constant \(k_{app}\) was 0.21 ± 0.02 min\(^{-1}\) in control cells, but 0.45 ± 0.05 min\(^{-1}\) in phosducin-expressing cells. At early times of stimulation, the cAMP levels were actually higher in the phosducin-expressing cells (Table II). Thus, in addition to an overall reduction of cAMP accumulation, phosducin appeared to cause an enhancement at early time points.

In order to probe the intracellular mechanisms of these effects, we measured cAMP accumulation in permeabilized cells. This was done to allow the introduction of various inhibitors of intracellular proteins into the cell interior. To simplify these assays, cAMP was determined at three different time points after addition of isoproterenol: after 20 s (higher levels of cAMP in phosducin-expressing cells), 2 min, and after 20 min (lower levels of cAMP in phosducin-expressing cells). Table II shows that the overall effects of phosducin were the same in digitonin-permeabilized cells. The absolute values of cAMP/cell were very similar in permeabilized and intact cells, and the early enhancement and the later inhibition caused by phosducin were also present, even though these effects were slightly smaller than in intact cells.

As a first step, phosducin antibodies were added to the permeabilized cells to verify the specificity of the observed effects. These antibodies essentially abolished the differences between control and phosducin-expressing cells (Table II, Fig. 3). This suggests that both the early enhancement and the late inhibi-
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**Isoproterenol-induced cAMP-accumulation in digitonin-permeabilized phosducin-expressing and control A431 cells**

Phosducin-expressing and control transfected cells were preincubated for 30 min with 100 μM of the phosphodiesterase inhibitor IBMX and were then, where indicated, permeabilized with digitonin. Phosducin antibodies (1:200 dilution) or the βARK inhibitors heparin (100 nM) or the βARK-inhibitor peptide KTAIAKFERLQTVTNYFITSE (50 μM) were added, 10 μM (~)-isoproterenol was added 10 min later, and cAMP levels were determined after the indicated periods of time always in the continued presence of 100 μM IBMX. Data are mean ± S.E.; n = 6; paired t test: *, p < 0.05; **, p < 0.01.

| Condition          | 20 s   | 40 s   | 60 s   | 2 min  | 20 min  |
|--------------------|--------|--------|--------|--------|---------|
| **Intact cells**   |        |        |        |        |         |
| Control            | 0.08 ± 0.02 | 0.23 ± 0.05 | 0.37 ± 0.03 | 1.05 ± 0.70 | 2.87 ± 0.08 |
| Phosducin          | 0.16 ± 0.01** | 0.35 ± 0.04** | 0.40 ± 0.04 | 0.70 ± 0.09* | 1.12 ± 0.14** |
| **Permeabilized cells** |        |        |        |        |         |
| Control            | 0.12 ± 0.02 | 0.19 ± 0.02** | 0.53 ± 0.07 | 0.75 ± 0.06* | 1.78 ± 0.19** |
| Phosducin antibody | 0.15 ± 0.02 | 0.14 ± 0.02 | 0.56 ± 0.03 | 2.34 ± 0.16 |         |
| Heparin            | 0.22 ± 0.02 | 0.20 ± 0.02 | 0.66 ± 0.04 | 2.15 ± 0.15 |         |
| Phosducin          | 0.20 ± 0.02 | 0.12 ± 0.02** | 0.61 ± 0.11 | 2.10 ± 0.26 |         |

**Fig. 3. Modulation of the effects of phosducin expression on cAMP accumulation in digitonin-permeabilized A431 cells by inhibitors.** cAMP accumulation in digitonin-permeabilized phosducin-expressing and control transfected cells was measured in the presence of phosducin antibodies, or the βARK inhibitors heparin (100 nM) or the βARK-inhibitor peptide KTAIAKFERLQTVTNYFITSE (50 μM). Phosducin effects were plotted as the ratio of the respective cAMP levels in phosducin-expressing and control cells as given in Table II. Open circles, control (no inhibitors); inverted triangles, phosducin antibodies; squares, heparin; upright triangles, βARK-inhibitor peptide. Data are means ± S.E. of six independent experiments with duplicate determinations.

The activity elicited by 10 μM isoproterenol was 27% of the activity in the presence of 100 μM forskolin, whereas in membranes from control cells it was 35%. Forskolin-stimulated activities were the same in membranes from the two cell lines (Fig. 4, legend), indicating that these effects of phosducin were exerted at the receptor/G-protein level.

Phosphorylation of phosducin by protein kinase A (PKA) has been shown to abolish the effects of phosducin on G-protein function (20, 38). This suggests that in our experiments enough unphosphorylated phosducin must have remained present in order to exert effects on cAMP accumulation. The peptide PKA-inhibitor PKI was added to permeabilized cells to determine whether inhibition of PKA activity would cause an enhancement of the effects of phosducin expression. Fig. 5 shows that PKI caused an exaggeration of the phosducin effects; it increased the initial enhancement of cAMP levels and led to greater inhibition of cAMP accumulation at later time points. These data indicate that phosphorylation of the expressed phosducin by endogenous PKA resulted in significant inhibi-
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**Fig. 4.** Desensitization of β2-adrenergic receptors in phosducin-expressing (panel B) and control (panel A) A431 cells. The β2-adrenergic receptors were desensitized by incubation of the intact cells with 10 μM (-)-isoproterenol for 10 min. Isoproterenol was then washed away at 4°C, crude membranes were prepared from the cells, and adenylyl cyclase activity was determined in the presence of the indicated concentrations of (-)-isoproterenol and was expressed as % of the activity in the presence of 100 μM forskolin. The latter activities were 280 ± 34 pmol cAMP/min/mg of protein in control, and 291 ± 35 pmol cAMP/min/mg of protein in phosducin-expressing cells, and were not significantly different in membranes from desensitized and from non-desensitized cells. Quantitative analysis of the control and desensitization curves as described under “Experimental Procedures” gave a desensitization of 77 ± 6% in control cells and 33 ± 13% in phosducin-expressing cells. Data are mean ± S.E. of four independent experiments with duplicate determinations.

**Fig. 5.** Effects of PKA inhibitors on isoproterenol-induced cAMP accumulation in digitonin-permeabilized phosducin-expressing and control A431 cells. Phosducin-expressing (phd) and control-transfected (con) cells were permeabilized as described in the legend to Fig. 3. The PKA inhibitor peptide (PKI, Sigma, 1 μM) was added, 10 μM (-)-isoproterenol was added 10 min later, and cAMP levels were determined after the indicated periods of time (always in the presence of 100 μM IBMX). Phosducin effects were plotted as the ratio of the respective cAMP levels in phosducin-expressing and control cells. Triangles, PKI; circles, control (from Fig. 3). The absolute values of cAMP (fmoles/cell) in the presence of PKI were: control cells, 20 ± 0.11 ± 0.01, 2 min 2.07 ± 0.19, 20 min 6.26 ± 0.19; phosducin-expressing cells, 20 ± 0.27 ± 0.02, 2 min 0.97 ± 0.14, 20 min 3.24 ± 0.32. Data are mean ± S.E. of six independent experiments with duplicate determinations.

is ~10% of its level in brain, and ~2% of that of Gβγ, which are presumed to represent the primary target of phosducin. Expression of phosducin had two apparently opposite effects: it inhibited cAMP accumulation in response to long-term (several minutes) β2-receptor stimulation, and it accelerated the cAMP response to short-term β2-receptor stimulation. The first effect, inhibition of β2-receptor-mediated cAMP-production, is analogous to the inhibitory effects of phosducin in reconstituted systems which were mentioned above. We assume that they are due to an interaction of phosducin with Gβγ, which results in inhibition of Gβγ activation and, consequently, in inhibition of Gβγ-mediated signaling (20). In fact, the reduction of cAMP levels seen here is similar to the extent of G-protein inhibition observed earlier (20). A small inhibitory effect of the phosducin could also be observed in the membranes prepared from these cells (Fig. 4), which is compatible with the presence of small amounts of phosducin in the membrane fraction.

The second effect, the initial acceleration of the cAMP response to isoproterenol, appears to be due to an inhibition of β2-adrenergic receptor desensitization. We have shown earlier that phosducin can inhibit receptor phosphorylation by βARK in reconstituted systems (15). This inhibition is thought to be due to the fact that phosducin competes with the kinase for the G-protein βγ-subunits which serve as membrane anchors for the kinase (39–41). Such an inhibition of βARK-mediated receptor phosphorylation should result in reduced βARK-mediated receptor desensitization. Since βARK-mediated desensitization of β2-adrenergic receptors in A431 cells occurs with a T1/2 of less than 15 s (36), the lack of such desensitization should be observed very soon after receptor stimulation. Indeed, the enhanced cAMP levels in phosducin-expressing cells were already seen 20 s after the addition of isoproterenol. Assessment of β2-adrenergic receptor desensitization in two-step assays is compatible with this hypothesis.

The two effects of phosducin combined result in a “sharpening” of the cAMP signal in response to isoproterenol. The initial acceleration in cAMP production followed by a reduced plateau result in a more rectangular response pattern which is reflected in a higher time constant of cAMP accumulation in the phosducin-expressing cells. These effects of phosducin are further complicated by the fact that they are antagonized by PKA-mediated phosphorylation of phosducin. It has been
shown that phosphorylation of phosducin by PKA can result in marked inhibition of phosducin’s effects in reconstituted systems (15, 20, 38). Phosducin in our A431 cells is apparently phosphorylated by endogenous PKA, since addition of PKI results in an enhancement of the effects of phosducin. However, inhibition of phosducin’s effects by PKA was obviously incomplete in these cells, since otherwise no long-term effects of phosducin would be observable. Fig. 6 shows a diagram illustrating the position that phosducin may have in feedback loops of G-protein-mediated signaling.

It is remarkable that phosducin at an expression level of ~1 pmol/mg of protein can inhibit G-protein-mediated signaling even though the level of G-protein βγ-subunits is about 50 times higher. This suggests that in some way phosducin may act preferentially on activated G-proteins, or that it interacts specifically with βγ-subunits which may be involved in βγ-adrenergic receptor/adenylyl cyclase signaling. Using a series of defined G-protein βγ-subunits we have not been able to detect major differences in their affinities for phosducin (42). The former hypothesis is supported by the observation that in a reconstituted retinal system the inhibitory effects of phosducin on G-protein-mediated signaling increased with time (21). This may be interpreted as evidence for a preferential interaction of phosducin with βγ-subunits which dissociate from α-subunits in response to receptor activation. Signal amplification at the receptor—G-protein step is only a fewfold in most hormonal systems (1, 2, 43). Thus, effective dampening of such responses would be expected to occur when the levels of a Gpγ-binding protein are similar to those of the respective receptors activated in response to stimuli.

Taken together our data indicate that phosducin at physiological levels has distinct effects on G-protein-mediated signaling in intact cells. Such effects would, depending on the expression level and their affinity for Gpγ, be common to all Gpγ-binding proteins. These proteins may thereby become additional components of G-protein systems and add another level of complexity to the many mechanisms regulating such systems.

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REFERENCES

1. Bourne, H. R., Sanders, D. A., and McCormick, F. (1990) Nature 348, 125–132
2. Hepler, J. R., and Gilman, A. G. (1992) Trends Biochem. Sci. 17, 383–387
3. Offermanns, S., and Schultz, G. (1994) Naunyn-Schmiedeberg’s Arch. Pharmacol. 355, 329–338
4. Hausdorff, W. P., Caron, M. G., and Lefkowitz, R. J. (1990) FASEB J. 4, 2881–2889
5. Lohse, M. J. (1993) Biochem. Biophys. Acta 1179, 171–188
6. Birnbaumer, L. (1992) Cell 71, 1069–1072
7. Neer, E. J. (1995) Cell 80, 249–257
8. Müller, S., and Lohse, M. J. (1995) Biochem. Soc. Trans. 23, 141–148
9. Musacchio, A., Gibson, T., Rice, P., Thompson, J., and Saraste, M. (1993) Trends Biochem. Sci. 18, 343–348
10. Macias, M. J., Musacchio, A., Penningth., Ï, Nilges, M., Saraste, M., and Maheshwari, H. (1994) Nature 369, 672–675
11. Yoon, H. S., Haiduk, P. J., Petros, A. M., Oleijniczak, E. T., Meadows, R. P., and Fesik, S. W. (1994) Nature 369, 672–675
12. Koch, W. J., Inglese, J., Stone, W. C., and Lefkowitz, R. J. (1993) J. Biol. Chem. 268, 8256–8260
13. Touhara, K., Inglese, J., Pitcher, J. A., Shaw, G., and Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 10217–10220
14. Chen, J., Devivo, M., Dingus, H., Le, T., and Lohse, M. J. (1995) J. Biol. Chem. 270, 120–124
15. Lohse, M. J., Hohlenm, P., Hohlenm, E. J. M., and Lohse, M. J. (1995) FASEB J. 9, 1179, 218–218
16. Lee, R. H., Ting, T. D., Lieberman, B. S., Rosenthal, R. A., and Lohse, M. J. (1995) J. Biol. Chem. 269, 6191–6197
17. Koch, W. J., Rockman, H., Samama, P., Hamilton, R. A., and Lohse, M. J. (1995) Science 269, 1350–1353
18. Lee, R. H., Lieberman, B. S., and Lohse, R. N. (1987) Biochemistry 26, 3983–3990
19. Reig, J. A., Yu, L., and Klein, D. C. (1990) J. Biol. Chem. 265, 5816–5824
20. Bauer, P. H., Pho, P., Puzich, M., Pippig, S., Hohlenm, E. J. M., and Lohse, M. J. (1992) Nature 358, 73–76
21. Lee, R. H., Ting, T. D., Lieberman, B. S., Tobisai, D. E., Lohse, R. N., and Ho, Y-K. (1992) J. Biol. Chem. 267, 25104–25112
22. Lohse, M. J. (1992) Naunyn-Schmiedeberg’s Arch. Pharmacol. 345, 444–451
23. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Harper, J. F., and Brooker, G. (1975) J. Cyclic Nucleotide Res. 1, 207–218
25. Lohse, M. J., Lefkowitz, R. J., and Benovic, J. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3011–3015
26. Lohse, M. J., Benovic, J. L., Lefkowitz, R. J., and Lohse, M. J. (1990) J. Biol. Chem. 265, 3210–3211
27. Winstel, R., Lohse, M. J., and Lohse, M. J. (1995) Naunyn-Schmiedeberg’s Arch. Pharmacol. 351, R39
28. Clark, R. B., Friedman, J., Johnson, J. A., and Kunkel, M. W. (1987) FASEB J. 1, 289–297
29. Pippig, S., Andexinger, S., Daniel, K., Puzich, M., Lohse, R. G., and Lohse, M. J. (1993) J. Biol. Chem. 268, 3201–3208
30. Hohlenm, P., Bekman, M., Elce, J. S., and Lohse, M. J. (1993) FEBS Lett. 324, 59–62
31. Benovic, J. L., Mayor, F., Jr., Staniszewski, C., Lefkowitz, R. J., and Caron, M. G. (1987) J. Biol. Chem. 262, 9026–9032
32. Sweeney, P. C., and Robishaw, J. D. (1984) J. Biol. Chem. 259, 13806–13813
33. Lohse, M. J., Lefkowitz, R. J., and Benovic, J. L. (1994) Mol. Pharmacol. 46, 1–9
34. Lohse, M. J. (1990) J. Biol. Chem. 265, 3210–3211
35. Black, J. W., and Lef, P. (1983) Proc. Soc. Roy. Lond. B 220, 141–162
36. Roth, N., Campbell, P. T., Caron, M. G., Lefkowitz, R. J., and Lohse, M. J. (1991) J. Biol. Chem. 266, 6707–6710
37. Yoshida, T., Willardson, B. M., Wilkins, J. F., Jensen, G. J., Thorburn, B. D., and Bittensky, M. W. (1985) J. Biol. Chem. 260, 24550–24557
38. Haga, K., and Haga, T. (1990) J. Biol. Chem. 265, 47–47
39. Haga, K., and Haga, T. (1992) J. Biol. Chem. 267, 2222–2227
40. Pitcher, J. A., Inglese, J., Higgin, J. B., Ariza, 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
41. Müller, S., Strath, A., Schröder, S., Bauer, P., and Lohse, M. J. (1996) J. Biol. Chem. 271, 11781–11786
42. Lohse, M. J., Klotz, K. N., and Schwabe, U. (1991) Mol. Pharmacol. 38, 517–523