Agonist-induced Internalization of the Platelet-activating Factor Receptor Is Dependent on Arrestins but Independent of G-protein Activation

ROLE OF THE C TERMINUS AND THE (D/N)PXXY MOTIF*

Received for publication, October 18, 2001
Published, JBC Papers in Press, November 29, 2001, DOI 10.1074/jbc.M110058200

Zhangguo Chen, Denis J. Dupré, Christian Le Gouill, Marek Rola-Pleszczynski, and Jana Staňková*
From the Immunology Division, Department of Pediatrics, Université de Sherbrooke, Sherbrooke, Québec J1H 5N4, Canada

As with most G-protein-coupled receptors, repeated agonist stimulation of the platelet-activating factor receptor (PAFR) results in its desensitization, sequestration, and internalization. In this report, we show that agonist-induced PAFR internalization is independent of G-protein activation but is dependent on arrestins and involves the interaction of arrestins with a limited region of the PAFR C terminus. In cotransfected COS-7 cells, both arrestin-2 and arrestin-3 could be coimmunoprecipitated with PAFR, and agonist stimulation of PAFR induced the translocation of both arrestin-2 and arrestin-3. Furthermore, coexpression of arrestin-2 with PAFR potentiated receptor internalization, whereas agonist-induced PAFR internalization was inhibited by a dominant negative mutant of arrestin-2. The coexpression of a minigene encoding the C-terminal segment of the receptor abolished PAF-induced arrestin translocation and inhibited PAFR internalization. Using C terminus deletion mutants, we determined that the association of arrestin-2 with the receptor was dependent on the region between threonine 305 and valine 330 because arrestin-2 could be immunoprecipitated with the mutant PAFRSTOP330 but not PAFRSTOP305. Consistently, stop330 could mediate agonist-induced arrestin-2 translocation, whereas stop305 could not. Two other deletion mutants with slightly longer regions of the C terminus, PAFRSTOP311 and PAFRSTOP317, also failed to induce arrestin-2 translocation. Finally, the PAFR mutant Y293A, containing a single substitution in the putative internalization motif DPXXY in the seventh transmembrane domain (which we had shown to be able to internalize but not to couple to G-proteins) could efficiently induce arrestin translocation. Taken together, our results indicate that ligand-induced PAFR internalization is dependent on arrestins, that PAFR can associate with both arrestin-2 and -3, and that their translocation involves interaction with the region of residues 318–330 in the PAFR C terminus but is independent of G-protein activation.

Cell responsiveness to agonists of G-protein-coupled receptors (GPCRs)¹ is usually characterized by a rapid desensitization to subsequent exposures, followed by a resensitization in the absence of stimulation (1–4). The internalization of GPCRs is believed to be responsible, at least in part, for desensitization and/or for resensitization (5–10) of many GPCRs, the prototype being the β2-adrenoreceptor (5, 6, 11–14). Members of the arrestin family play an important role in the process of GPCR endocytosis (15–17). After agonist-activation, the receptors are phosphorylated by GPCR-specific kinases (GRKs) and second messenger-dependent kinases (18). For the β2-adrenoreceptor and other GPCRs, agonist stimulation leads to arrestin recruitment to the plasma membrane and binding with the phosphorylated receptor. Phosphorylation and arrestins prevent coupling of the receptor to the heterotrimeric G-protein and are believed to be the key elements of a rapid desensitization (19–21). Arrestins play an adaptor role by targeting the receptor to clathrin-coated pits and by binding the heavy chain of clathrin as well as the adaptor molecule AP2 (15, 16, 22). In addition, arrestins may facilitate the action of dynamin by recruiting c-Src (17, 23), which is necessary for its phosphorylation (23, 24). The internalized receptor is subsequently either dephosphorylated and recycled to the plasma membrane or targeted for degradation.

Platelet-activating factor (PAF) is a potent proinflammatory phospholipid mediator involved in the physiology and pathology of a variety of systems, including central nervous, reproductive, respiratory, and immune responses (25, 26). PAF binds to a specific receptor (PAFR), which is a member of the GPCR family (25–31). PAFR couples to several second messenger systems that include phospholipid turnover through phospholipase (PL) Cγ and PLCβ activation (32–35), MAP kinase activation (32, 36), and the JAK/STAT pathway (37). By using the techniques of ligand binding, immunostaining, inositol phosphate (IP) production, and intracellular calcium concentration measurement, we and others have demonstrated that PAFR is also subject to desensitization, internalization, and resensitization (9, 33, 38, 39). Furthermore, it has been shown by us and others (9, 39) that the intracellular C terminus of PAFR plays

¹ The abbreviations used are: GPCR, G-protein-coupled receptor; GFP, green fluorescent protein; GRKs, GPCR-specific kinases; PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; CHO, Chinese hamster ovary; WT, wild type; IP, inositol phosphate; PBS, phosphate-buffered saline; RIPA, radioimmune precipitation buffer; STAT, signal transducers and activators of transcription; MAP, mitogen-activated protein; MAPK, MAP kinase; DMEM, Dulbecco’s modified Eagle’s medium; AEBSF, 4-(2-aminoethyl)-benzenesulfonyl fluoride; TLCK, Na-p-tosyl-lysyl chloromethyl ketone.
an important role in receptor internalization, but its association with arrestins has not been shown. The molecular mechanism through which PAFR is desensitized, internalized, and desensitized has not yet been fully elucidated.

The aim of the current study is to demonstrate whether nonvisual arrestins interact with PAFR and participate in receptor internalization after agonist stimulation. Our results indicated that PAFR internalization was regulated by arrestin-2 and -3 and that the C terminus of PAFR contained arrestin-2 or -3 binding sites and contributed to arrestin translocation after PAF stimulation. In addition, the DPXXY motif in the seventh transmembrane domain of PAFR may be involved in maintaining the proper receptor conformation responsible for arrestin translocation and colocalization with PAFR. Finally, agonist-induced PAFR internalization and arrestin recruitment were independent of G-protein signaling.

**EXPERIMENTAL PROCEDURES**

**Materials**—Reagents were obtained from the following sources: oligonucleotides were synthesized at Invitrogen, *Pfu* polymerase was from Roche Molecular Biochemicals, restriction endonucleases and modifier enzymes were from Promega and Amersham Biosciences, bovine serum albumin from Sigma-Aldrich, *FLAG* Transfection reagent from Roche, platelet-activating factor from Calbiochem, anti-GFP antibody from CLONTECH (Palo Alto, CA), and a-PAFR monoclonal antibody from Roche Molecular Biochemicals, 50% (w/v) bovine serum albumin. Binding reactions were carried out on 0.1% (w/v) bovine serum albumin. Binding reactions were carried out on 0.1% (w/v) bovine serum albumin. Binding reactions were carried out on 0.1% (w/v) bovine serum albumin. Binding reactions were carried out on 0.1% (w/v) bovine serum albumin.

**RESULTS**

**Functional Characterization of Mutant Receptors**—The mutants of PAFR used in this report are illustrated in Fig. 1. The PAFR-GFP fusion protein was constructed in order to follow PAFR internalization in real time, and the C terminus deletion mutants were constructed in order to define the region of arrestin and GRK binding. Fig. 2 shows that in 10 M PAF, the reactions were terminated by the addition of perchloric acid. Inositol phosphates were extracted and separated on Dowex AG1-X8 (Bio-Rad) columns. Total labeled inositol phosphates were then counted by liquid scintillation.

**Inositol Phosphate Determination**—COS-7 cells transfected with indicated cDNAs were labeled for 18–24 h with [3H]myo-inositol (Amerham Biosciences) at 3 Ci/ml in DMEM (high glucose, without inositol, from Invitrogen). After labeling, cells were washed and preincubated for 5 min in PBS at 37 °C. The PBS was removed, and cells were incubated in DMEM containing 0.1% bovine serum albumin and 20 mM LiCl for 5 min, then stimulated for 10 min with 10-9 M PAF. The reactions were terminated by the addition of perchloric acid. Inositol phosphates were then extracted and separated on Dowex AG1-X8 (Bio-Rad) columns. Total labeled inositol phosphates were then counted by liquid scintillation.

**Cell Culture and Transfection**—COS-7, CHO, and HEK293 cells were grown in Dulbecco's modified Eagle's medium high glucose (DMEM), Dulbecco's modified Eagle's medium F12, and minimum essential medium (MEM), respectively, supplemented with 10% fetal bovine serum and 100 μg/ml garamycin. For confocal microscopy, cells were seeded in 6-well plates for a coverslip at a density of 1 × 106 cells/well. Cells were transfected following manufacturer's instructions using FuGENEX transfection reagent. Cells were fixed with 2% paraformaldehyde for 5 min at 37 °C for the indicated time, and then lysed with 0.5 ml of Radioimmunoprecipitation assay buffer, resolved by SDS-PAGE, and subjected to Western blot analysis. Inositol phosphates were then counted by liquid scintillation.

**RESULTS**

**Functional Characterization of Mutant Receptors**—The mutants of PAFR used in this report are illustrated in Fig. 1. The PAFR-GFP fusion protein was constructed in order to follow PAFR internalization in real time, and the C terminus deletion mutants were constructed in order to define the region of arrestin and GRK binding. The stop311, Asp289, and Tyr293 mutants have been described previously (39, 40). The binding characteristics of PAFR-GFP and stop311 were examined in transiently transfected COS-7 cells using WEB2086, a PAFR receptor antagonist, (Fig. 2) and showed that both mutant receptors had an affinity comparable with that of wild-type PAFR. Response to PAF as measured by inositol phosphate production was similar to WT in both PAFR-GFP and stop311 (results not illustrated). Stop305, on the other hand, showed significantly reduced binding and IP production, ~10% of WT (results not illustrated). In this study, we used four different cell types (COS-7, CHO, HEK293, and RBL-2H3) to assure ourselves of the universality of the studied mechanisms. Experiments were done with all the cell types, but only one is illustrated for each experiment.

**Internalization of PAFR Is Dependent on Arrestins**—We had shown that the human PAFR is internalized via clathrin-coated vesicles (39). We have now investigated whether internalization was associated with arrestins. Fig. 3A shows that in COS-7 cells transfected with PAFR-GFP cDNA coexpression of arrestin-2 potentiated internalization after PAF stimulation,
whereas a dominant negative mutant of arrestin-2 inhibited internalization. When cells were observed in real time using a temperature-controlled chamber, the overexpression of arrestin-2 in HEK293 cells also potentiated internalization. Fig. 3B shows that in cells transfected with PAFR-GFP the receptor was partially internalized within 30 min, but when arrestin-2 was coexpressed the receptor was completely internalized within this time frame. In addition, the receptor was internalized in much larger vesicles than without arrestin coexpression. The same phenomena were observed when the cells were cotransfected with arrestin-3 (results not illustrated).

Association of Arrestins with PAFR—We coexpressed WT PAFR and arrestin-2-GFP or arrestin-3-GFP in COS-7 cells, immunoprecipitated the receptor, and showed that both arrestin-2 and arrestin-3 could immunoprecipitate with the WT receptor (Fig. 4A). In CHO cells cotransfected with PAFR and arrestin-2-GFP cDNAs, PAF induced a redistribution of arrestin-2 from the cytosol to the cellular membrane within 5 min; by 30 min, both the receptor and arrestin colocalized to discrete intracytoplasmic vesicles (Fig. 4B). This contrasted with much more discrete internalization of the PAFR when cotransfected with only the GFP protein (Fig. 4B, lower left panel). Similar results were seen with arrestin-3-GFP cotransfection (results not illustrated).

Because our results indicated that arrestins were necessary for PAFR internalization, we used certain mutants of PAFR that showed different internalization properties to verify whether they could communoprecipitate arrestin-2. COS-7 cells were cotransfected with arrestin-2 and either WT or mutant PAFR or pcDNA3. The cell lysates were subjected to immunoprecipitation with anti-c-Myc antibodies and immunoblotted with anti-arrestin-2 antibodies, followed by anti-PAFR polyclonal antibodies to verify the levels of PAFR. The total cell lysates were blotted with anti-arrestin-2 antibodies to verify equivalent arrestin expression in all samples. The results indicate that the 25 amino acids between residues 305 and 330 of the receptor are essential for arrestin association (Fig. 5). Because we had shown that this region was also, at least in part, involved in the internalization of the receptor, we used a mu-
tant receptor which does not internalize but has a mutation that is not in the C terminus. The mutant D289A, which is impaired in its internalization potential, failed to coimmunoprecipitate arrestin-2, whereas the mutant D289N (39), which internalizes normally, coimmunoprecipitated with arrestin-2 comparably to WT (Fig. 5).

Ligand-induced Translocation of Arrestins Is Independent of G-protein Coupling—We next examined whether mutant PAFRs that did not internalize and/or were not coupled to G-proteins could induce the translocation of arrestins. Both mutants D289A and Y293A are not coupled to G-proteins but, unlike D289A, Y293A internalizes comparably to WT (39). The conservative substitutions of these residues D289N and Y293F produce receptors that are both coupled to G-proteins and internalize. Fig. 6 shows that after PAF stimulation all mutants induced arrestin-2-GFP translocation to the membrane with the exception of D289A. These results support the hypothesis that arrestins are necessary for PAFR internalization but that G-protein coupling is not necessary for arrestin translocation.

The role of the C terminus of PAFR in the redistribution of arrestins was examined with C terminus deletion mutants. COS-7 cells were cotransfected with arrestin-2-GFP and PAFR WT or stop mutant cDNAs, and redistribution of arrestin-2-GFP was examined after a 20 min stimulation with PAF. Fig. 7 shows that stop305, stop311, and stop317 did not induce membrane translocation of arrestins, in contrast to the stop330 mutant, which induced arrestin translocation similarly to the WT. These results indicate that the portion of the C terminus between residues 318 and 330 is necessary for PAFR association with and translocation of arrestins (Figs. 5 and 7, respectively).

To study the role of the C terminus of PAFR without using truncated mutants, we constructed a minigene that expressed residues 300 to 342 from the C terminus. The C terminus...
transfection, the cells were stimulated with 10 μM PAF for 20 min, immunostained with anti-c-Myc antibody, and examined by confocal microscopy. The results are representative of four independent experiments.

blocked PAF-induced PAFR-GFP internalization when cotransfected with the receptor in RBL-2H3 cells (Fig. 8A). In COS-7 cells, C terminus cotransfection prevented the PAF-induced cell-surface loss of receptors, both in the presence and absence of overexpressed arrestin-3 (Fig. 8B) or arrestin-2 (results not illustrated).

PAF-induced translocation of arrestin-2-GFP was also inhibited by C terminus coexpression, as was the formation of distinct internalization vesicles (Fig. 9). On the other hand, IP production was enhanced by C terminus coexpression with the PAFR in COS-7 cells, indicating that not all functions of the receptor were negatively affected by the minigene. Interestingly, the coexpression of arrestin-3 with PAFR decreased PAF-induced IP production by ~50%, and this decrease was antagonized by the coexpression of the C terminus (Fig. 10), further supporting the data that arrestins interact with the C-terminal region of the receptor.

DISCUSSION

In the present study we have shown that PAFR induces the translocation of nonvisual arrestins, which is independent of G-protein signaling and correlates with the internalization potential of PAFR mutants. In addition, a dominant negative mutant of arrestin-2 inhibited PAF-induced PAFR internalization as did the expression of the C-terminal tail of the receptor, which also inhibited arrestin translocation, again indicating the positive correlation between PAFR internalization and arrestin translocation.

In the vast family of G-protein-coupled receptors, a diversity of internalization pathways, or lack thereof, has been described. Many GPCRs induce the translocation of arrestins and subsequently colocalize with them in intracellular compartments (10), whereas other GPCRs induce the translocation of arrestins but do not colocalize intracellularly after internalization (41). Still other receptors will internalize without mobilizing arrestins but can use the arrestin system if arrestins are overexpressed (41).

In the cell systems used, arrestin-2-GFP was distributed in the cytosol and nucleus, whereas arrestin-3-GFP was only found in the cytosol, in unstimulated cells, as had been observed previously (42). We observed ligand-dependent translocation of both arrestin-2 and arrestin-3 to the plasma membrane and their colocalization in intracellular vesicles with PAFR. This is in agreement with the internalization of many GPCRs but contrasts with others, such as the adenosine A2B receptor that promotes the translocation of both arrestin-2 and-3 but colocalizes only with arrestin-2 in endosomes (43). The β2-adrenoreceptor and the 5-HT2A receptor also induce arrestin translocation but do not colocalize with them intracellularly (42, 44).

The kinetics of arrestin-2- or -3-GFP translocation in COS-7 and CHO cells were similar (data not illustrated), whereas the formation and internalization of larger vesicles containing arrestin-2 or -3-GFP and PAFR varied greatly among the different cell types. In COS-7 cells, most PAFR and arrestins were colocalized on the plasma membrane after 20 min of PAF treatment, with few internalized vesicles in the cytosol. In contrast, in CHO cells vesicle formation was initiated after 2 min of PAF stimulation, and almost all PAFR and arrestins were internalized in punctate vesicles after 20 min. In the absence of coexpressed arrestins, the internalization of PAFR in COS-7, CHO, and HEK293 cells was into discrete vesicles, whereas in RBL-2H3 cells the vesicles were large and resembled those found in the other cell types after coexpression of arrestins. The differences might be a reflection of the different quantities of arrestins and GRKs normally expressed in the various cell types (45). Comparably, CXCR1-GFP internalization in HEK293 cells requires coexpression of both GRK-2 and arrestin-2, whereas in RBL-2H3 cells no exogenous proteins were needed because these two cell lines express different amounts of endogenous arrestin-2 and GRK-2 (46).

There is little consensus on the role of internalization motifs in GPCRs. The motif DPXY, which exists in the seventh transmembrane domain of PAFR, is believed to contribute to internalization of some (2, 47), but not all, (48, 49) receptors. In PAFR, changing aspartate 289 into alanine (D289A mutant) practically abolishes receptor internalization, whereas substitution of the amino acid with asparagine (D289N mutant) has no impact on internalization (39). Our current results are consistent with our previous observation in that D289A did not induce arrestin translocation, whereas D289N did. The DPXXY motif might therefore be responsible for maintaining a proper receptor conformation necessary to lead to arrestin translocation rather than directly interacting with arrestins (50).

The intracellular second and third loops, in addition to the C terminus of GPCRs, have all been shown to interact with arrestins. For some receptors, the intracellular loops are important for interaction with arrestins (51–54); for some, it is the C terminus (55–60), and for others, both the loops and the C terminus may interact with arrestins but with different affinities (61). Our previous results demonstrated that truncation of the C terminus of the human PAFR from cysteine 317 abrogated receptor internalization (39). Ishii et al. (9) showed that the C terminus of guinea pig PAFR was also involved in receptor desensitization and internalization. The aforementioned studies, however, did not address the molecular mechanisms of PAFR desensitization and internalization. The current results obtained from confocal microscopy on live and fixed cells provide direct evidence that the C terminus-truncated mutants of PAFR were not able to induce arrestin translocation and colocalization with the receptor after PAF stimulation. In addition, the immunoprecipitation results showed that the mutant lack-
ing the major part of the C terminus could not coimmunoprecipitate arrestins, unlike the wild type.

Our results, using PAFR mutants, implicate the coordinate action of a distal motif (DP\textsubscript{XY}) with a direct binding site in the C terminus. Mutations in the distal motif altered PAFR conformation, and this change resulted in the uncoupling of the receptor from G-proteins. Interestingly, the Y293A mutant, which could not couple to G-proteins, was still capable of mediating arrestin translocation and coimmunoprecipitation. Our findings also show that residue Asp-289 of the motif is responsible for a conformation that influences two aspects of receptor function, G-protein coupling and arrestin recruitment, given that the mutation of this residue to alanine resulted in the loss of both functions. It is noteworthy that changing the Asp-289 to Asn-289 fully maintained functions of PAFR in terms of G-protein coupling, internalization, induction of arrestin translocation, and association with the receptor. The conformation maintained by the motif DP\textsubscript{XY} requires the special structure supplied by aspartate or asparagine, which is lost in the D289A substitution. It is also possible that arrestins associate with PAFR through a domain constituted of both DP\textsubscript{XY} and C terminus motifs.

Arrestins have been shown to have the capacity to recruit and bind kinases from the MAPK pathway and the Src family (62, 63). It is as yet unclear, however, whether arrestin translocation is signal driven, but our results indicate that the translocation of arrestins after agonist stimulation can be independent of G-protein signaling. We have recently shown that the PAFR can signal through Tyk2, a member of the Janus family of tyrosine kinases (JAK), in the absence of G-protein activation (37), which may indicate that other signaling pathways of GPCRs may bypass the G-protein requirement.
PAFR-induced Arrestin Translocation Is G-protein Independent

In summary, the current study demonstrates for the first time that nonvisual arrestins are required for PAFR internalization after agonist stimulation and that both the C terminus and the internalization motif of the receptor play crucial roles in arrestin translocation and colocalization. Moreover, signaling through G-proteins is not required for agonist-induced arrestin recruitment and PAFR trafficking.

Acknowledgments—We thank Denis Dingras, Sylvie Turcotte, and Léonid Volkov for excellent technical advice and collaboration.

REFERENCES

1. Goldstein, J. L., Brown, M. S., Anderson, R. G., Russell, D. W., and Schneider, W. J. (1985) Annu. Rev. Cell Biol. 1, 1–39
2. pearse, B. M., and Robinson, M. S. (1990) Eur. J. Biochem. 190, 283–292
3. Trowbridge, I. S. (1991) Curr. Opin. Cell Biol. 3, 1–8
4. Smythe, E., and Warren, G. (1991) Curr. Opin. Cell Biol. 3, 433–439
5. Pippig, S., Andexinger, S., and Lohse, M. J. (1995) Mol. Pharmacol. 48, 783–789
6. Krueger, K. M., Daaka, Y., Pitcher, J. A., and Lefkowitz, R. J. (1997) J. Biol. Chem. 272, 5–8
7. Zhang, J., Barak, L. S., Winkler, K. E., Caron, M. G., and Ferguson, S. S. (1997) J. Biol. Chem. 272, 7005–7014
8. Volga, O., Bogatkiewicz, G. S., Wisnie, C., Krummerfel, P., Jakobs, H. K., and van Koppen, C. J. (1998) J. Biol. Chem. 273, 12155–12160
9. Ishii, I., Saito, E., Izumi, T., Us, M., and Shimizu, T. (1998) J. Biol. Chem. 273, 9878–9885
10. Oakley, R. H., Laporte, S. A., Holt, J. A., Barak, L. S., and Caron, M. G. (1999) J. Biol. Chem. 274, 32248–32257
11. Yu, S. S., Lefkowitz, R. J., and Hausdorff, W. P. (1997) J. Biol. Chem. 272, 530–538
12. von Zastrow, M., and Kohlika, B. K. (1992) J. Biol. Chem. 267, 3530–3538
13. von Zastrow, M., and Kohlika, B. K. (1994) J. Biol. Chem. 269, 18448–18452
14. Ferguson, S. S., Menard, L., Barak, L. S., Koch, W. J., Celagianen, A. M., and Caron, M. G. (1995) J. Biol. Chem. 270, 24782–24789
15. 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
16. Laporte, S. A., Oakley, R. H., Holt, J. A., Barak, L. S., and Caron, M. G. (2000) J. Biol. Chem. 275, 23120–23126
17. Luttrell, L. M., Ferguson, S. S., Daaka, Y., Miller, W. E., Maudeley, S., Della Rocca, G. J., Lin, F., Kawaihatsu, H., Owada, K., Luttrell, D. K., Caron, M. G., and Barak, L. S. (1999) J. Biol. Chem. 274, 16287–16294
18. Barak, L. S., Tiberi, M., Freedman, N. J., Kwatra, M. M., Lefkowitz, R. J., and Caron, M. G. (1994) J. Biol. Chem. 269, 2790–2795
19. Hunyadi, L., Mor, B., Baulik, A. J., Ballia, T., and Catt, K. J. (1995) J. Biol. Chem. 270, 16622–16629
20. Leflavor, S. A., Servant, G., Richard, D. E., Escher, E., Guillelmette, G., and Ledre, R. (1996) Mol. Pharmacol. 49, 89–95
21. Le, K. B., Ptaszynski, J., and Rola-Pleszczynski, M., Gurevich, V. V., and Hasey, M. M. (2000) J. Biol. Chem. 275, 9284–9289
22. Van Koppen, C. J., Lenz, W., Junes, P. J., Zhang, C., Schmidt, M., and Jakobs, K. H. (1995) Eur. J. Biochem. 234, 536–541
23. Jockers, R., Da Silva, A. S., Strobarg, A. D., Bouvier, M., and Marullo, S. (1996) J. Biol. Chem. 271, 9355–9362
24. Lameh, P., Mahir, M., Sharma, Y. K., More, O., Ramachandran, J., and Sadee, W. (1992) J. Biol. Chem. 267, 13406–13412
25. Bennett, T. A., Maestas, D. C., and Prossnitz, E. R. (2000) J. Biol. Chem. 275, 24590–24594
26. Nussenzweig, D. R., Heinfink, M., and Gershengorn, M. C. (1993) J. Biol. Chem. 268, 2389–2392
27. Rodriguez, M. C., Xie, Y. B., Wang, H., Collison, K., and Segaloff, D. L. (1992) Mol. Endocrinol. 6, 327–336
28. Houtsman, E., Octave, J. N., and Maloteaux, J. M. (1996) Mol. Pharmacol. 49, 365–372
29. Thomas, W. G., Baker, K. M., Motel, T. J., and Thelkumkaru, T. J. (1995) J. Biol. Chem. 270, 22153–22159
30. Benyo, R. V., Fathi, Z., Battey, J. F., and Jensen, R. T. (1993) J. Biol. Chem. 268, 20285–20290
31. Parker, E. M., Swigart, P., Fengnally, M. H., Perkins, J. P., and Ross, E. M. (1995) J. Biol. Chem. 270, 6482–6487
32. Chen, Z. J., Zhao, J., Sun, Y., Hu, W., Li, Y., Cen, B., Wu, G. X., and Pei, G. (2000) J. Biol. Chem. 275, 2479–2485
33. McDonald, P. H., Chow, C. W., Miller, W. E., Laporte, S. A., Field, M. E., and Benson, J. L. (1995) J. Biol. Chem. 270, 1574–1577
34. Penela, P., Eforaza, A., Sarnago, S., and Mayor, F. (2001) EMBO J. 20, 5129–5138
Agonist-induced Internalization of the Platelet-activating Factor Receptor Is Dependent on Arrestins but Independent of G-protein Activation: ROLE OF THE C TERMINUS AND THE (D/N)PXXY MOTIF
Zhangguo Chen, Denis J. Dupré, Christian Le Gouill, Marek Rola-Plesczynski and Jana Stanková

J. Biol. Chem. 2002, 277:7356-7362.
doi: 10.1074/jbc.M110058200 originally published online November 29, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M110058200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 63 references, 46 of which can be accessed free at
http://www.jbc.org/content/277/9/7356.full.html#ref-list-1