Differential Regulation of Formyl Peptide and Platelet-activating Factor Receptors

ROLE OF PHOSPHOLIPASE Cβ3 PHOSPHORYLATION BY PROTEIN KINASE A*

Hydar Ali‡§, Silvano Sozzani‡§, Ian Fisher‡, Alastair J. Barr‡, Ricardo M. Richardson‡, Bodduluri Haribabu‡, and Ralph Snyderman§

From the Departments of ‡Medicine and §Immunology, Duke University Medical Center, Durham, North Carolina 27710

© 1998 by The American Society for Biochemistry and Molecular Biology, Inc.

Printed in U.S.A.

Formylated peptides (e.g. n-formyl-Met-Leu-Phe (fMLP)) and platelet-activating factor (PAF) mediate chemotactic and cytotoxic responses in leukocytes through receptors coupled to G proteins that activate phospholipase C (PLC). In RBL-2H3 cells, fMLP utilizes a pertussis toxin (ptx)-sensitive G protein to activate PLC, whereas PAF utilizes a ptx-insensitive G protein. Here we demonstrate that fMLP, but not PAF, enhanced intracellular cAMP levels via a ptx-sensitive mechanism. Protein kinase A (PKA) inhibition by H-89 enhanced inositol phosphate formation stimulated by fMLP but not PAF. Furthermore, a membrane-permeable cAMP analog 8-(4-chlorophenylthio)-cAMP (cpt-cAMP) inhibited phosphoinositide hydrolysis and secretion stimulated by fMLP but not PAF. Both cpt-cAMP and fMLP stimulated PLCβ3 phosphorylation in intact RBL cells. The purified catalytic subunit of PKA phosphorylated PLCβ3 immunoprecipitated from RBL cell lysate. Pretreatment of intact cells with cpt-cAMP and fMLP, but not PAF, resulted in an inhibition of subsequent PLCβ3 phosphorylation by PKA in vitro. These data demonstrate that fMLP receptor, which couples to a ptx-sensitive G protein, activates both PLC and cAMP production. The resulting PKA activation phosphorylates PLCβ3 and appears to block the ability of Gαi to activate PLC. Thus, both fMLP and PAF generate stimulatory signals for PLCβ3, but only fMLP produces a PKA-dependent inhibitory signal. This suggests a novel mechanism for the bidirectional regulation of receptors which activate PLC by ptx-sensitive G proteins.

Many extracellular signaling molecules including neurotransmitters, hormones, and chemoattractants mediate their biological responses via the activation of G protein-coupled receptors through stimulation of adenyl cyclase, phospholipase C (PLC), and ion channels (1). Continuous agonist stimulation leads to waning of the biological response by a process termed desensitization (2). Receptor phosphorylation by G protein-coupled receptor kinases as well as by second messenger-activated kinases, such as protein kinase A (PKA) and protein kinase C, are important in receptor desensitization (2, 3). Additionally, chemoattractant responses are regulated at the level of PLC (4). Chemoattractants such as formyl peptides (e.g., n-formyl-Met-Leu-Phe (fMLP)), the anaphylatoxin C5a, and interleukin-8 activate PLC by a ptx-sensitive G protein, likely Gα12 (5, 6). The chemoattractant receptor for PAF couples to both ptx-sensitive and -insensitive G proteins. The latter, Gαq, likely activates PLCβ by a different mechanism (7, 8).

fMLP and PAF receptors have been shown to display differences in susceptibility to desensitization (9). This laboratory has developed methodology to study the regulation of chemoattractant receptors in the leukocyte-like RBL-2H3 (RBL) cell line (10–13). Using this model, it was found that a membrane permeable cAMP analog caused inhibition of Ca2+ mobilization stimulated by fMLP but not PAF (4). This difference could be potentially related to the distinct G protein usage of these receptors. The present study characterizes this observation and demonstrates that fMLP causes an increase in cAMP production both in neutrophils and transfected RBL cells and that the resulting PKA activation leads to inhibition of a biological response, secretion. In addition, the data show that PLCβ3 is a direct substrate for phosphorylation by PKA and that fMLP receptor-stimulated PLCβ3 phosphorylation by PKA provides a previously unrecognized mechanism for the counter regulation of cellular activation.

EXPERIMENTAL PROCEDURES

Materials—[32P]Orthophosphate (8500–9120 Ci/mmol), myo-[2-3H(N)]inositol (24.4 Ci/mmol), [γ-32P]ATP (6000 Ci/mmol), and [γ-32P]GTP (6000 Ci/mmol) were purchased from NEN. fMLP, PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine), and the protein kinase A inhibitor H-89 were purchased from Sigma. Affinity-purified polyclonal antibody against PLCβ3 was obtained from Santa Cruz Biotechnology. Pertussis toxin and all tissue culture reagents were purchased from Life Technologies, Inc. The catalytic subunit of PKA was obtained from Promega. The Radiomunassay kit for cAMP measurement was purchased from Amersham Corp.

Cell Culture and Assays—RBL cells stably expressing epitope-tagged fMLP and PAF receptors were used throughout this study (4, 10, 11, 14). Cell culture, neutrophil purification, GTPase activity, phosphoinositide hydrolysis, Ca2+ mobilization, secretion, and in vivo PLCβ3 phosphorylation were performed exactly as described by us previously (4, 9, 10). HL-60 cells were differentiated with 1.3% dimethyl sulfoxide (2, 3). Cell culture, neutrophil purification, and GTPase activity were performed as described by us previously (4, 9, 10). HL-60 cells were differentiated with 1.3% dimethyl sulfoxide for 5–6 days. In vitro phosphorylation of PLCβ3 was performed essentially as described for PLCβ3 (15). Briefly, cells (5 × 106) were treated with various agents or buffer for 5 min in the presence of isobutyl-

ptx, pertussis toxin; IBMX, isobutylmethylxanthine.
methylxanthine (IBMX) (400 μM), lysed, and immunoprecipitated with anti-PLCβ3 antibody. The immune complex was washed with a buffer containing 40 mM Tris-HCl, (pH 7.4), MgOAc (20 mM), ATP (20 μM) and resuspended in the same buffer (50 μl) supplemented with 2 μCi of [γ-32P]-ATP. Phosphorylation was started via the addition of 1 μl of purified PKA. The reaction was stopped by adding 1 ml of ice-cold buffer, and the immune complex was washed three times. The proteins were resolved on a 6% SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. For cAMP assay, cells (0.5–1.0 × 10^6/ml) were preincubated for 10 min with 400 μM IBMX and stimulated with fMLP, C5a, or PAF. The reactions were quenched, and cAMP measurements were carried out as described in the cAMP kit manual.

RESULTS

Differential Regulation of fMLP- and PAF-mediated Phosphoinositide Hydrolysis and Secretion by cAMP—RBL cells were preincubated with or without a membrane-permeable cAMP analog, cpt-cAMP (1 μM; 5 min) and dose responses of fMLP- and PAF-stimulated phosphoinositide hydrolysis and degranulation were determined. As shown in Fig. 1A, cpt-cAMP caused a substantial inhibition of fMLP-stimulated generation of inositol phosphates. In contrast, PAF-mediated phosphoinositide hydrolysis was inhibited by only ~30% (Fig. 1B). Furthermore, cpt-cAMP substantially inhibited secretion stimulated by fMLP but had no effect on the response to PAF (Fig. 1C and D). The half-maximal and maximal concentrations of cpt-cAMP for inhibition of fMLP-stimulated responses were ~0.1 μM and ~1 μM, respectively (Fig. 1E and F). Cpt-cAMP also caused a substantial inhibition of intracellular Ca^{2+} mobilization stimulated by fMLP but not PAF (4). To test whether cAMP also inhibited responses to other chemoattractant receptors that activate PLC via a ptx-sensitive G protein, its effect on C5a-stimulated Ca^{2+} mobilization in RBL cells was tested. In the absence of cpt-cAMP, stimulation with C5a (1 nM) resulted in an increase of intracellular mobilization of 162 ± 5.6 nM (n = 4) over basal. In the presence of cpt-cAMP (1 μM, 5 min), this response was reduced to 27 ± 2.2 nM (83% inhibition).

The ability of fMLP and PAF to produce cAMP was also determined. As shown in Fig. 2A, fMLP caused a ~2.5-fold increase in cAMP over basal, whereas PAF produced no response. Treatment of cells with ptx (100 ng/ml, overnight) resulted in a complete inhibition of fMLP-stimulated cAMP generation. To determine whether the cAMP increase caused by chemoattractants has a regulatory effect on their cellular responses, cells were preincubated with the PKA inhibitor H-89 and its effect on fMLP and PAF-stimulated generation of inositol phosphates was tested. H-89 pretreatment resulted in a 2.5-fold increase in fMLP-stimulated generation of inositol phosphates (Fig. 2B). In contrast, the response to PAF was enhanced only by ~20% and this effect was lost in cells treated with ptx. It was determined whether the findings in transfected RBL cells occurred in human neutrophils and in neutrophil-like HL-60 cells. As shown in Fig. 3A, fMLP caused a significant increase in cAMP generation in neutrophils and in dimethyl sulfoxide differentiated HL-60 cells. C5a also stimulated cAMP production in neutrophils (Fig. 3A). PAF did not stimulate cAMP formation in either neutrophils or HL-60 cells. Cpt-cAMP caused a substantial inhibition of fMLP but not PAF-stimulated Ca^{2+} mobilization in both cell types (Fig. 3B). Cpt-cAMP also caused a substantial inhibition of C5a-stimulated Ca^{2+} mobilization in neutrophils (Fig. 3B).

Effect of cAMP on fMLP- and PAF-mediated GTPase Activity—To determine the effect of cpt-cAMP on G protein activation, RBL cells were treated with buffer or cpt-cAMP, then membranes were prepared, and the ability of fMLP and PAF to stimulate GTPase activity was measured. Both fMLP and PAF
Phosphorylation of PLCβ3 by PKA

Fig. 2. Generation of cAMP and effect of H-89 on fMLP- and PAF-stimulated generation of inositol phosphates in RBL cells. A, for cAMP generation, cells were cultured overnight in the absence and presence of ptx (100 ng/ml). The following day, cells (0.5 × 10^6/ml) were preincubated with IBMX (400 μM; 10 min) and stimulated with fMLP (100 nM) and PAF (100 nM). Reactions were quenched after 5 min, and intracellular cAMP concentrations were determined. B, cells were preincubated with H-89 (30 μM for 10 min) and stimulated with fMLP (30 nM) or PAF (3 nM), reactions were quenched 10 min later, and the generation of [3H]inositol phosphates ([3H]IPs) was determined. For PAF-stimulated responses, cells were also treated with ptx (100 ng/ml (PAF, + ptx) and preincubated with or without H-89. Basal levels of 728 ± 64 and 992 ± 38 cpm in the absence and presence of H-89, respectively, were subtracted from the values shown. Ptx treatment had no significant effect on the basal level. Data are presented as mean ± S.E. of one of three experiments performed in triplicate.

Fig. 3. Generation of cAMP and effect of cpt-cAMP on fMLP-, C5a-, and PAF-stimulated Ca^{2+} mobilization in human neutrophils and HL-60 cells. A, cells (0.5 × 10^6/ml) were preincubated with IBMX (400 μM; 10 min) and stimulated with fMLP (100 nM), C5a (10 nM), or PAF (100 nM). Reactions were quenched after 5 min, and intracellular CAMP concentrations were determined. B, indo-1-loaded neutrophils and HL-60 cells were preincubated with cpt-cAMP (1 mM; 5 min) and then stimulated with fMLP (0.3 nM), C5a (0.3 nM), or PAF (0.3 nM), and intracellular Ca^{2+} mobilization was determined. Values are the mean ± S.E. of three experiments. *p < 0.05 compared with the response in the absence of chemotactic agents. Numbers in the parentheses indicate percent inhibition of response by cpt-cAMP.

stimulated GTPase activity in a dose-dependent manner in membranes from buffer or cpt-cAMP-treated cells showing that cpt-cAMP pretreatment had no effect on this PAF- or fMLP-stimulated response (data not shown).

In Vivo and In Vitro Phosphorylation of PLCβ3—Using antibodies that specifically recognize different PLCβ isoforms, it was shown that of the known PLCβ isoforms only PLCβ3 is expressed in RBL cells (4). To determine if other PLCβ isoforms are expressed in RBL cells at levels below the detection of antibodies, specific oligonucleotide primers for different PLCβ isoforms were used for reverse transcriptase-polymerase chain reaction on RNA from RBL cells. Rat brain RNA was used as a control. PLCβ1 was the only PLCβ isoform detected in RBL cells (data not shown).

fMLP and cpt-cAMP caused a dose-dependent phosphorylation of PLCβ3 (Fig. 4, A and B). To determine whether PLCβ3 was a substrate for PKA, cell lysates were immunoprecipitated with anti-PLCβ3 antibody and the ability of purified catalytic subunit of PKA to phosphorylate PLCβ3 was tested in the presence of [γ-32P]ATP. As shown in Fig. 4C, PLCβ3 phosphorylation by PKA was detected within 1 min and remained elevated for 15 min. In vitro PLCβ3 phosphorylation by PKA was blocked in immunoprecipitates prepared from cells treated with cpt-cAMP (Fig. 4D). To determine whether fMLP-stimulated PLCβ3 phosphorylation was in part activated by PKA, whole cells were incubated with either fMLP or PAF, PLCβ3 was immunoprecipitated, and the ability of PKA to phosphorylate the enzyme was determined. Treatment of cells with fMLP but not PAF resulted in a substantial inhibition of PLCβ3 phosphorylation by PKA (Fig. 4D).

DISCUSSION

Chemotactic, microbicidal, and cytotoxic effects of phagocytic leukocytes are stimulated by chemotactants such as formylated peptides and PAF via the G protein-coupled receptor activation of PLC (16). The ability of fMLP to produce a transient increase in cAMP production in neutrophils is well documented (17–20); however, the physiological effects of this phenomenon were not known. A recent study did demonstrate that a PKA inhibitor enhanced superoxide production stimulated by fMLP in human neutrophils (21), but the mechanism of this effect was also unknown. It also remained to be determined whether PAF stimulated cAMP production in neutrophils and whether inhibition of PKA resulted in the regulation of PAF-mediated biological responses as well. The present work utilizing RBL cells stably expressing fMLP and PAF receptors demonstrated that fMLP, but not PAF, caused an increase in cAMP formation and that preincubation of cells with a membrane permeable cAMP analog resulted in inhibition of both phosphoinositide hydrolysis and exocytotic release of granules stimulated by fMLP. Furthermore, the PKA inhibitor H-89 enhanced fMLP-stimulated phosphoinositide hydrolysis. These data suggest that cAMP produced by fMLP provides a mechanism for counter regulation of an fMLP-stimulated biological response, secretion via the inhibition of PLC activation. This phenomenon appears to be specific for fMLP versus PAF, as the latter did not cause cAMP generation nor did exogenously added cAMP inhibit PAF-induced phosphoinositide hydrolysis or secretion. This difference in the regulation of fMLP- and PAF-mediated responses in transfected RBL cells is likely to be physiologically relevant as similar differences in the generation
In guinea pig neutrophils, fMLP greatly potentiates cAMP production stimulated by PKA. The mechanism by which fMLP causes an increase in cAMP is not well understood. The finding that phosphodiesterase inhibitors which cause a decrease in fMLP-stimulated cAMP increase is sufficient to counter regulate the response to fMLP suggests that cAMP formation is mediated via the modification of PLC. The ability of cpt-cAMP to inhibit fMLP-induced phosphoinositide hydrolysis and secretion is likely mediated via the phosphorylation of PLCβ3 by PKA. Importantly, the finding that fMLP stimulated the formation of cAMP and that pretreatment of cells with cAMP resulted in a partial inhibition of PKA-stimulated PLCβ3 phosphorylation in vitro indicate that fMLP-stimulated PLCβ3 phosphorylation is mediated, at least in part, by PKA. The ability of the PKA inhibitor H-89 to enhance fMLP-stimulated inositol phosphates generation suggests that counter regulation of fMLP-stimulated biological responses is likely mediated via the PKA-induced phosphorylation of PLCβ3. This form of inhibition appears to be specific for fMLP versus PAF, which did not stimulate cAMP formation and did not block PLCβ3 phosphorylation by PKA in vitro. Furthermore, the PKA inhibitor H-89 had no effect on the ptx-insensitive component of PAF-mediated generation of inositol phosphates.

The demonstration that cAMP did not cause phosphorylation of fMLP receptor (11) and had no effect on fMLP-stimulated GTPase activity indicates that its ability to block fMLP-stimulated inositol phosphate generation and secretion is not mediated at the level of the receptor or its coupling to G protein. Inhibition of membrane inositol phospholipid resynthesis and thus a reduction in the availability of substrate for PLC has been postulated as a mechanism by which cAMP inhibits fMLP-stimulated generation of inositol phosphates in human neutrophils (31). This mechanism is unlikely because cAMP did not inhibit phosphoinositide hydrolysis stimulated by PAF in RBL cells, and it had no effect on PAF-induced Ca2+ mobilization in human neutrophils and HL-60 cells. The selective inhibition of fMLP response by cAMP is therefore likely to be mediated via the modification of PLC. Using reverse transcriptase-polymerase chain reaction (this study) and Western blotting with PLCβ isoform-specific antibodies (4) it was shown that PLCβ3 is the only known PLCβ isozyme expressed in RBL cells. Furthermore, both fMLP and cpt-cAMP caused phosphorylation of PLCβ3 in this cell line. In addition, purified catalytic subunit of PKA phosphorylated PLCβ3 immunoprecipitated from an RBL cell lysate. The observation that preincubation of cells with cpt-cAMP blocked subsequent in vitro PLCβ3 phosphorylation by PKA suggests that PLCβ3 is a direct substrate for PKA. The ability of cpt-cAMP to inhibit fMLP-induced phosphoinositide hydrolysis and secretion is likely mediated via the phosphorylation of PLCβ3 by PKA. Importantly, the finding that fMLP stimulated the formation of cAMP and that pretreatment of cells with cAMP resulted in a partial inhibition of PKA-stimulated PLCβ3 phosphorylation in vitro indicate that fMLP-stimulated PLCβ3 phosphorylation is mediated, at least in part, by PKA. The ability of the PKA inhibitor H-89 to enhance fMLP-stimulated inositol phosphates generation suggests that counter regulation of fMLP-stimulated biological responses is likely mediated via the PKA-induced phosphorylation of PLCβ3. This form of inhibition appears to be specific for fMLP versus PAF, which did not stimulate cAMP formation and did not block PLCβ3 phosphorylation by PKA in vitro. Furthermore, the PKA inhibitor H-89 had no effect on the ptx-insensitive component of PAF-mediated generation of inositol phosphates.
Receptors that couple to \(G_s\) and cause an elevation of intracellular cAMP levels are known to inhibit PLC-mediated responses to other receptors that couple to ptx-sensitive G protein (15). The data herein suggest that the mechanism for this phenomenon may be through the phosphorylation of PLC\(\beta_3\) by PKA at a site which blocks G\(p_s\)-mediated activation. PLC\(\beta\) other than PLC\(\beta_3\) may also be regulated by PKA. For example, phosphorylation of PLC\(\beta_2\) by PKA has been suggested as a mechanism by which cAMP inhibits fMLP-stimulated phosphoinositide hydrolysis in differentiated HL-60 cells (15). It is, however, important to note that the neutrophil-like HL-60 cells express both PLC\(\beta_2\) and PLC\(\beta_3\) (4). Furthermore, cAMP causes phosphorylation of PLC\(\beta_3\) in the human monocyte-like U937 cells and the murine macrophage-like J774.1 cells (4). The finding that fMLP-stimulated phosphoinositide hydrolysis and Ca\(^{2+}\) mobilization were not completely blocked in neutrophils isolated from mice deficient in PLC\(\beta_2\) suggests that both PLC\(\beta_2\) and PLC\(\beta_3\) are activated by fMLP (35). Unlike PLC\(\beta_2\), which is expressed only in certain cells of hematopoietic origin, PLC\(\beta_3\) is expressed in many cell types and tissues (32, 33). Therefore, cross-talk between the adenylyl cyclase and PLC pathways is likely to be mediated via the phosphorylation of PLC\(\beta_2\), PLC\(\beta_3\), or both depending on the cell type on which the receptors are expressed.

REFERENCES
1. Rens-Domiano, S., and Hamn, H. E. (1995) FEBS Lett. 9, 1059–1066
2. Dohlman, H. G., Thorner, J., Caron, M. G., and Lefkowitz, R. J. (1991) Annu. Rev. Biochem. 60, 653–688
3. Steffel, R. H., Pitcher, J. A., and Lefkowitz, R. J. (1997) J. Membr. Biol. 157, 1–8
4. Ali, H., Fisher, L., Haribabu, B., Richardson, R. M., and Snyderman, R. (1997) J. Biol. Chem. 272, 11706–11709
5. Polakos, P. G., Uehing, R. J., and Snyderman, R. (1988) J. Biol. Chem. 263, 4969–4976
6. Wu, D., LaRosa, G. J., and Simon, M. I. (1993) Science 261, 101–103
7. Vergheese, M. W., Charles, L., Jakoi, L., Dillon, S. B., and Snyderman, R. (1987) J. Immunol. 138, 4374–4380
8. Amatruda, T. T., III, Gerard, N. P., Gerard, C., and Simon, M. I. (1993) J. Biol. Chem. 268, 10139–10144
9. Tomhave, E. D., Richardson, R. M., Didsbury, J. R., Menard, L., Snyderman, R., and Ali, H. (1994) J. Immunol. 153, 3267–3275
10. Ali, H., Richardson, R. M., Tomhave, E. D., Didsbury, J. R., and Snyderman, R. (1993) J. Biol. Chem. 268, 24247–24254
11. Ali, H., Richardson, R. M., Tomhave, E. D., DuBose, R. A., Haribabu, B., and Snyderman, R. (1994) J. Biol. Chem. 269, 24557–24563
12. Richardson, R. M., Ali, H., Tomhave, E. D., Haribabu, B., and Snyderman, R. (1995) J. Biol. Chem. 270, 27829–27833
13. Richardson, R. M., DuBose, R. A., Ali, H., Tomhave, E. D., Haribabu, B., and Snyderman, R. (1995) Biochemistry 34, 14193–14201
14. Richardson, R. M., Haribabu, B., Ali, H., and Snyderman, R. (1996) J. Biol. Chem. 271, 28717–28724
15. Liu, M. Y., and Simon, M. I. (1996) Nature 382, 83–87
16. Snyderman, R., and Uehing, R. J. (1992) in Inflammation: Basic Principles and Clinical Correlates (Gallin, J. J., Goldstein, I. M., and Snyderman, R. eds) pp. 412–439, Raven Press, New York
17. Simeowitz, L., Fischhein, L. C., Spilberg, I., and Atkinson, J. P. (1980) J. Immunol. 124, 1482–1491
18. Verghese, M. W., Fox, K., McPhail, L. C., and Snyderman, R. (1985) J. Biol. Chem. 260, 6769–6775
19. Dai, Y., Holgate, S. T., Church, M. K., and Shute, J. K. (1994) J. Leukoc. Biol. 56, 776–783
20. Spisani, S., Paresechi, M. C., Buzzi, M., Colamussi, M. I., Biondi, C., Tranjello, S., Pagani Zecchini, G., Pagliaudanga Paradisi, M., Torrini, I., and Ferretti, M. E. (1996) Cell. Signalling 8, 269–277
21. Mitsuoka, T., Takeshige, K., Furuno, T., Tanaka, T., Hidaka, K., Abe, M., and Hara, N. (1995) Mol. Cell. Biochem. 145, 19–24
22. Schudt, C., Winder, S., Forderkunz, S., Hatzelmann, A., and Ulrich, V. (1991) Naunyn-Schmiedebergs Arch. Pharmacol. 344, 682–690
23. Federman, A. D., Conklin, B. R., Schrader, K. A., Reed, R. R., and Bourne, H. R. (1992) Nature 356, 159–161
24. Choi, E. J., Xia, Z., Villacres, E. C., and Storm, D. R. (1993) Curr. Opin. Cell Biol. 5, 269–273
25. Tauszig, R., Tang, W. J., Hepler, J. R., and Gilman, A. G. (1994) J. Biol. Chem. 269, 6995–7000
26. Prezont, R. T., Matsouka, I., Mattei, M. G., Pouille, Y., Defer, N., and Hanoune, J. (1996) J. Biol. Chem. 271, 13900–13907
27. Suzuki, T., Hazeki, O., Hazeki, K., U., M., and Katada, T. (1996) Biochem. Biophys. Acta 1263, 72–78
28. Uehing, R. J., Gettys, T. W., Tomhave, E., Snyderman, R., and Didsbury, J. R. (1992) Biochem. Biophys. Res. Commun. 183, 1033–1039
29. Tsu, R. C., Lai, H. W., Allen, R. A., and Wong, Y. H. (1995) Biochem. J. 309, 331–339
30. Tsu, R. C., Allen, R. A., and Wong, Y. H. (1995) Mol. Pharmacol. 4, 835–841
31. Kato, H., Ishitoya, J., and Takenawa, T. (1986) J. Biol. Chem. 261, 13900–13907
32. Hanoune, J. (1996) J. Biol. Chem. 221, 12424–12431
33. Smrcka, A. V., and Sternweis, P. C. (1993) J. Biol. Chem. 268, 9667–9674
34. Banzo, Y., Nakashima, S., Ohzawa, M., and Nokaza, Y. (1996) J. Biol. Chem. 271, 14989–14994
35. Jiang, H. P., Kuang, Y. N., Wu, Y. P., Xie, W., Simon, M. I., and Wu, D. Q. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7971–7975