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Abstract

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 PLCβ_3 phosphorylation in intact RBL cells. The purified catalytic subunit of PKA phosphorylated PLCβ_3 immunoprecipitated from RBL cell lysate. Pretreatment of intact cells with cpt-cAMP and fMLP, but not PAF, resulted in an inhibition of subsequent PLCβ_3 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 PLCβ_3 and appears to block the ability of G($\beta\gamma$) to activate PLC. Thus, both fMLP and PAF generate stimulatory signals for PLCβ_3, 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.

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Differential Regulation of Formyl Peptide and Platelet-activating Factor Receptors

ROLE OF PHOSPHOLIPASE Cβ_3 PHOSPHORYLATION BY PROTEIN KINASE A*

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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 PLCβ_3 phosphorylation in intact RBL cells. The purified catalytic subunit of PKA phosphorylated PLCβ_3 immunoprecipitated from RBL cell lysate. Pretreatment of intact cells with cpt-cAMP and fMLP, but not PAF, resulted in an inhibition of subsequent PLCβ_3 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 PLCβ_3 and appears to block the ability of $G_{\beta\gamma}$ to activate PLC. Thus, both fMLP and PAF generate stimulatory signals for PLCβ_3, 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 adenylyl cyclase, phospholipase C (PLC),[1] 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 PLCβ by releasing $\beta\gamma$ subunits ($G_{\beta\gamma}$) of a pertussis toxin (ptx)-sensitive G protein, likely $G_{i\alpha 2}$ (5, 6). The chemoattractant receptor for PAF couples to both ptx-sensitive and -insensitive G proteins. The latter, $G_{\alpha q}$, 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 Ca^{2+} 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 PLCβ_3 is a direct substrate for phosphorylation by PKA and that fMLP receptor-stimulated PLCβ_3 phosphorylation by PKA provides a previously unrecognized mechanism for the counter regulation of cellular activation.

EXPERIMENTAL PROCEDURES

Materials—[^{32}P]Orthophosphate (8500–9120 Ci/mmol), *myo*-[2-^{3}H(N)]inositol (24.4 Ci/mmol), [γ-^{32}P]ATP (6000 Ci/mmol), and [γ-^{32}P]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 Calbiochem. Recombinant C5a and cpt-cAMP were purchased from Sigma. Affinity-purified polyclonal antibody against PLCβ_3 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 Radioimmunoassay 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, Ca^{2+} mobilization, secretion, and *in vivo* PLCβ_3 phosphorylation were performed exactly 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 PLCβ_3 was performed essentially as described for PLCβ_2 (15). Briefly, cells (5×10^6) were treated with various agents or buffer for 5 min in the presence of isobutyl-

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[1] The abbreviations used: PLC, phospholipase C; fMLP, *n*-formyl-Met-Leu-Phe; PAF, platelet-activating factor; G protein, GTP-regulatory protein; RBL, rat basophilic leukemia; cpt-cAMP, 8-(4-chlorophenylthio)-adenosine 3′:cyclic monophosphate; PKA, protein kinase A; ptx, pertussis toxin; IBMX, isobutylmethylxanthine.

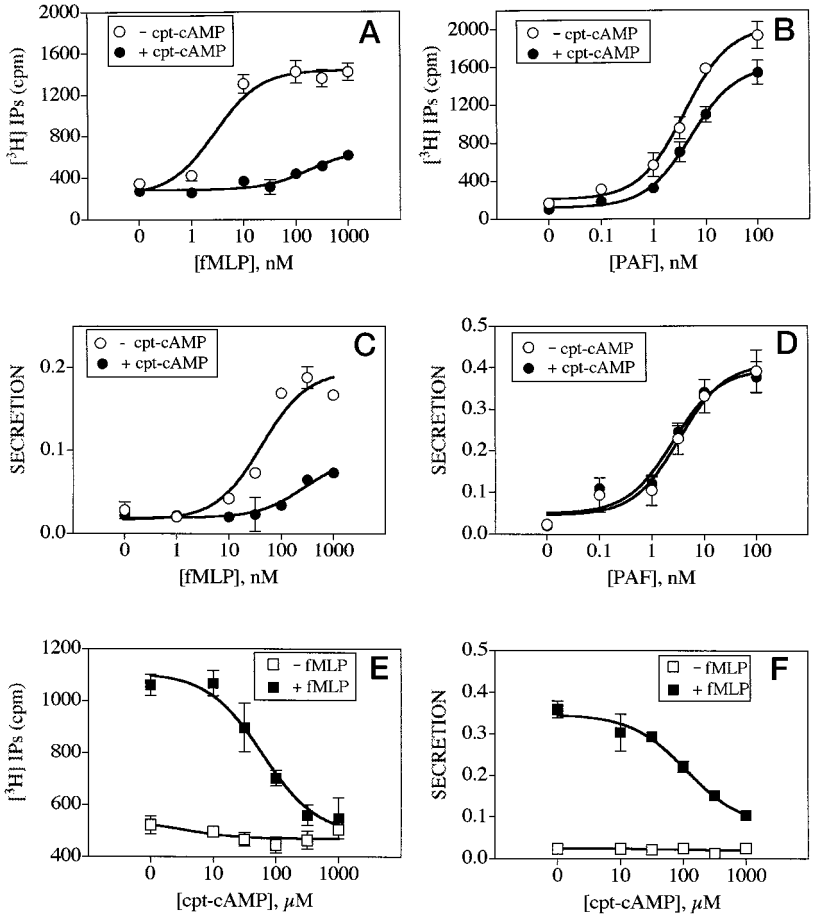

FIG. 1. **Effects of cpt-cAMP on fMLP- and PAF-mediated generation of [^{3}H]inositol phosphates and release of β-hexosaminidase.** For phosphoinositide hydrolysis (*A*, *B*, and *E*), RBL cells were cultured overnight in the presence of [^{3}H]inositol (2 μCi/ml) in an inositol-free medium. For secretion (*C*, *D*, and *F*), cells were cultured in the same medium in the absence of [^{3}H]inositol. The following day, cells were washed in a HEPES-buffered saline containing 10 mM LiCl and preincubated with buffer or cpt-cAMP (1 mM) for 5 min, then stimulated with different concentrations of fMLP (*A* and *C*) or PAF (*B* and *D*), and the generation [^{3}H]inositol phosphates (*[^{3}H]IPs*) and the release of β-hexosaminidase (secretion) were determined. Cells were also preincubated with different concentrations of cpt-cAMP for 5 min then left unstimulated (*−fMLP*) or stimulated with 10 nM fMLP (*+fMLP*), and the generation of (*E*) [^{3}H]IPs and (*F*) secretion were determined. Data are presented as mean ± S.E. of one of three experiments performed in triplicate.

methylxanthine (IBMX) (400 μM), lysed, and immunoprecipitated with anti-PLCβ_3 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^{32}P-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 mM; 5 min) and dose responses of fMLP- and PAF-stimulated phosphoinositide hydrolysis and degranulation were determined. As shown in Fig. 1*A*, 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. 1*B*). Furthermore, cpt-cAMP substantially inhibited secretion stimulated by fMLP but had no effect on the response to PAF (Fig. 1, *C* and *D*). The half-maximal and maximal concentrations of cpt-cAMP for inhibition of fMLP-mediated responses were ~0.1 mM and ~1 mM, respectively (Fig. 1, *E* 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 an increase of intracellular mobilization of 162 ± 5.6 nM (n = 4) over basal. In the presence of cpt-cAMP (1 mM, 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. 2*A*, 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 were tested. H-89 pretreatment resulted in a 2.5-fold increase in fMLP-stimulated generation of inositol phosphates (Fig. 2*B*). 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. 3*A*, 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. 3*A*). 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. 3*B*). Cpt-cAMP also caused a substantial inhibition of C5a-stimulated Ca^{2+} mobilization in neutrophils (Fig. 3*B*).

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

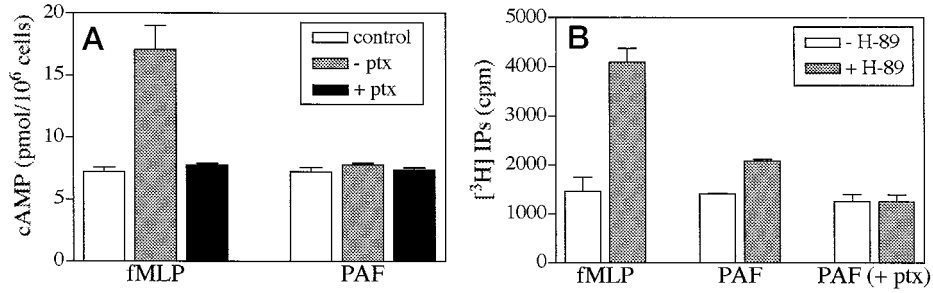

FIG. 2. **Generation of cAMP and effect of H-89 on fMLP- and PAF-stimulated generation of inositol phosphates in RBL cells.** *A*, for cAMP generation, cells were cultured overnight in the absence and presence of ptx (100 ng/ml). The following day, cells (0.5×10^6/ml were preincubated with IBMX (400 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 [^{3}H]inositol phosphates (*[^{3}H]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.

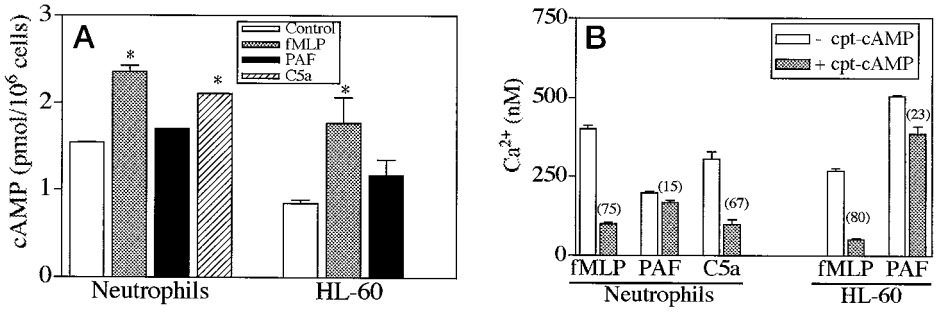

FIG. 3. **Generation of cAMP and effect of cpt-cAMP on fMLP-, C5a-, and PAF-stimulated Ca^{2+} mobilization in human neutrophils and HL-60 cells.** *A*, cells (0.5×10^6/ml) were preincubated with IBMX (400 μM; 10 min) and stimulated with fMLP (100 nM), C5a (10 nM), or PAF (100 nM). Reactions were quenched 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 chemoattractants. 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 PLCβ$_3$ 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. PLCβ$_3$ was the only PLCβ isoform detected in RBL cells (data not shown).

fMLP and cpt-cAMP caused a dose-dependent phosphorylation of PLCβ$_3$ (Fig. 4, *A* and *B*). To determine whether PLCβ$_3$ was a substrate for PKA, cell lysates were immunoprecipitated with anti-PLCβ$_3$ antibody and the ability of purified catalytic subunit of PKA to phosphorylate PLCβ$_3$ was tested in the presence of [γ-^{32}P]ATP. As shown in Fig. 4*C*, PLCβ$_3$ phosphorylation by PKA was detected within 1 min and remained elevated for 15 min. *In vitro* PLCβ$_3$ phosphorylation by PKA was blocked in immunoprecipitates prepared from cells treated with cpt-cAMP (Fig. 4*D*). To determine whether fMLP-stimulated PLCβ$_3$ phosphorylation was in part activated by PKA, whole cells were incubated with either fMLP or PAF, PLCβ$_3$ 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 PLCβ$_3$ phosphorylation by PKA (Fig. 4*D*).

DISCUSSION

Chemotactic, microbiocidal, and cytotoxic effects of phagocytic leukocytes are stimulated by chemoattractants 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

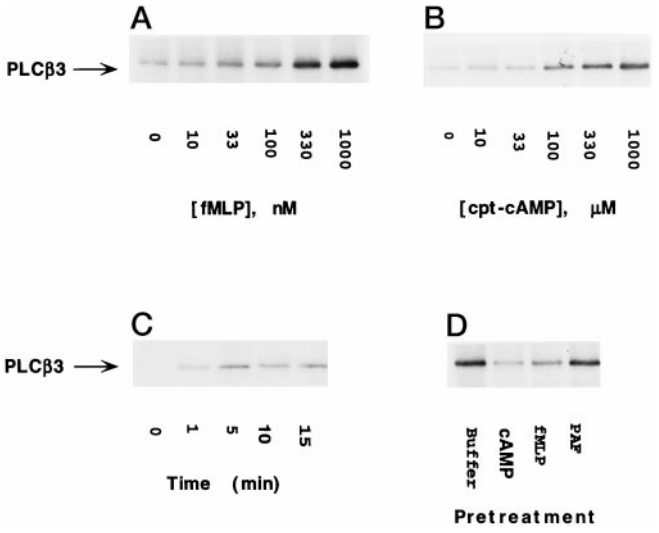

FIG. 4. **Phosphorylation of PLCβ_3.** ^{32}P- Labeled RBL cells were stimulated with different concentrations of *A*, fMLP and *B*, cpt-cAMP for 5 min. Cells were lysed, immunoprecipitated with anti-PLCβ_3 antibody, and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. *C*, RBL cells (5×10^6) were lysed and immunoprecipitated with anti-PLCβ_3 antibody. The immune complex (50 μl) was incubated with PKA (1 μl) in the presence of [γ-^{32}P]ATP. The phosphorylation reaction was quenched at different times via the addition of ice-cold radioimmune precipitation buffer. *D*, cells were preincubated with IBMX (400 μM) for 10 min and treated with buffer, cpt-cAMP (cAMP, 1 mM), fMLP (100 nM), or PAF (100 nM) for 5 min. Cells were lysed and immunoprecipitated with anti-PLCβ_3 antibody, and *in vitro* phosphorylation of PLCβ_3 was performed. The samples were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Results shown are from one of three similar experiments.

of cAMP and the regulation of cellular functions by cAMP were observed in the present study in human neutrophils and neutrophil-like HL-60 cells. These differences in the functional regulation of chemoattractant receptors are likely a consequence of G protein usage as suggested by the distinct ptx sensitivity of fMLP *versus* PAF receptors, with the former being sensitive and the latter is at least partially resistant (8, 10, 11).

In the studies reported here, fMLP stimulated a 40–50% increase in cAMP over basal levels in neutrophils and HL-60 cells (Fig. 3*A*). These data are consistent with previous findings from this and other laboratories (17–19). Furthermore, the ability of a PKA inhibitor to enhance fMLP-stimulated superoxide generation in neutrophils (21) suggests that the small fMLP-stimulated cAMP increase is sufficient to counter regulate the response to fMLP. This contention is supported by the finding that phosphodiesterase inhibitors which cause ~50% increase of cellular cAMP also result in a substantial inhibition of fMLP-stimulated superoxide release in neutrophils (22). The mechanism by which fMLP causes an increase in cAMP is not known (18, 23). Of the nine adenylyl cyclases identified, activation of types I and III are inhibited by all three forms of G_i proteins (24–26). In contrast, types II and IV are activated by G_s and $G_{\beta\gamma}$ of ptx-sensitive G proteins in a synergistic manner (23). In guinea pig neutrophils, fMLP greatly potentiates cAMP production stimulated by prostaglandin E_1 receptor and this enhancement is totally inhibited by ptx (27). In HEK 293 cells, which endogenously expresses adenylyl cyclase type III, fMLP causes an inhibition of cAMP production (28, 29). This inhibitory effect of fMLP likely results from the interaction of $G_{i\alpha}$ with type III adenylyl cyclase. However, in the same cell line transiently expressing adenylyl cyclase type II, fMLP causes the stimulation of cAMP production and this response is inhibited by ptx (30). This indicates that fMLP can either stimulate or inhibit cAMP formation depending on the subtype of adenylyl cyclase expressed. The observation that fMLP caused cAMP formation in RBL cells and that this response was completely inhibited by ptx suggest that $G_{\beta\gamma}$ directly interacts with adenylyl cyclases types II or IV to stimulate cAMP production. This contention is supported by the finding that PAF, which utilizes a ptx insensitive mechanism to cause intracellular Ca^{2+} mobilization in RBL cells, HL-60 cells and neutrophils, did not cause cAMP production in any of these cell types (Figs. 2*A* and 3*A*).

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 Ca^{2+} 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 PLCβ_3 is the only known PLCβ isozyme expressed in RBL cells. Furthermore, both fMLP and cpt-cAMP caused phosphorylation of PLCβ_3 in this cell line. In addition, purified catalytic subunit of PKA phosphorylated PLCβ_3 immunoprecipitated from an RBL cell lysate. The observation that preincubation of cells with cpt-cAMP blocked subsequent *in vitro* PLCβ_3 phosphorylation by PKA suggests that PLCβ_3 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 PLCβ_3 by PKA. Importantly, the finding that fMLP stimulated the formation of cAMP and that pretreatment of cells with fMLP resulted in a partial inhibition of PKA-stimulated PLCβ_3 phosphorylation *in vitro* indicate that fMLP-stimulated PLCβ_3 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 PLCβ_3. This form of inhibition appears to be specific for fMLP *versus* PAF, which did not stimulate cAMP formation and did not block PLCβ_3 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 data presented herein revealed that fMLP activated both PLCβ_3 and cAMP production via a ptx-sensitive pathway. Interestingly, PKA activated by this mechanism phosphorylated PLCβ_3 and blocked the subsequent activation of PLC by fMLP. It is likely that fMLP activates PLCβ_3 through the release of G$\beta\gamma$ (8, 10, 11). Thus the PKA-mediated phosphorylation of PLCβ_3 can be hypothesized to selectively block activation by $G_{\beta\gamma}$ as opposed to $G_{\alpha q}$, which is activated by PAF. This suggests a novel selective counter regulatory pathway for certain G protein-coupled receptors such as those for chemoattractants that activate a ptx-sensitive signaling pathway. Other receptors such as the α2-adrenergic, dopamine D_2 and adenosine A_1 receptors also activate both adenylyl cyclase and PLC (23). Given that PLCβ_3 is expressed abundantly in many cells and tissues such as platelets, leukocytes, brain, testes, and lung (32–34), phosphorylation of PLCβ_3 by PKA may be a novel

mechanism for the counter regulation of some but not other G protein-coupled receptors.

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 PLCβ_3 by PKA at a site which blocks $G_{\beta\gamma}$-mediated activation. PLCβ other than PLCβ_3 may also be regulated by PKA. For example, phosphorylation of PLCβ_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 PLCβ_2 and PLCβ_3 (4). Furthermore, cAMP causes phosphorylation of PLCβ_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 PLCβ_2 suggests that both PLCβ_2 and PLCβ_3 are activated by fMLP (35). Unlike PLCβ_2, which is expressed only in certain cells of hematopoietic origin, PLCβ_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 PLCβ_2, PLCβ_3, or both depending on the cell type on which the receptors are expressed.

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