Chemoattractants Stimulate Phosphatidylinositol-4-phosphate Kinase in Human Polymorphonuclear Leukocytes*

(Received for publication, July 19, 1989)

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Chemoattractant receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP$_2$) by phospholipase C is instrumental for leukocyte activation. Previous studies have demonstrated that chemoattractant treatment of intact polymorphonuclear leukocytes (PMN) causes a transient decrease in PIP$_2$ due to phospholipase C activation, followed by an increase in cellular PIP$_2$ levels. The present study determined whether chemoattractants altered the activities of the two enzymes responsible for the synthesis of PIP$_2$, phosphatidylinositol kinase, and phosphatidylinositol 4-phosphate (PIP) kinase. Incubation of intact PMN with the N-formylated peptide chemoattractant formyl-methionyl-leucyl-phenylalanine at 37°C caused a rapid (3 min), 2-fold stimulation of PIP kinase activity isolated from a particulate membrane fraction. The increase in PIP kinase was dose-dependent for a variety of N-formylated chemoattractants as well as leu-kotriene B$_4$. Lineweaver-Burk analysis showed that the $V_{\text{max}}$ of PIP kinase was increased 2-fold by formylmethionyl-leucyl-phenylalanine, without a significant change in the apparent $K_{\text{m}}$ of the enzyme for ATP. Phosphatidylinositol kinase was, however, not altered by any chemoattractants tested. Nonchemotactic activators of the oxidative burst in leukocytes such as phorbol myristate acetate and ionophore A23187 did not significantly alter PIP kinase, suggesting a specificity for chemotactic agents. These findings demonstrate direct, chemoattractant-induced stimulation of PMN PIP kinase which may serve to replenish the important phospholipid, PIP$_2$, in the membrane following its hydrolysis by phospholipase C.

The influx of calcium into human polymorphonuclear leukocytes (PMN)$^1$ is a prerequisite for chemoattractant-induced activation of inflammatory functions such as chemotaxis, lysosomal enzyme release, and superoxide anion generation (1–3). Chemoattractant receptor-mediated increases in leukocyte intracellular calcium concentrations are thought to result as a consequence of the hydrolysis of membrane phosphoinositides such as phosphatidylinositol 4,5-bisphosphate (PIP$_2$) into inositol 1,4,5-trisphosphate and diacylglycerol (4–6). The former molecule releases calcium directly from intracellular stores (7–10). Protein kinase C activation results from the stimulating actions of both diacylglycerol and calcium (11, 12), a process thought to be required for superoxide anion generation in human PMN (13, 14).

The molecular reservoir of these second messenger molecules, PIP$_2$, is formed by the phosphorylation of phosphatidylinositol (PI) and phosphatidylinositol 4-monophosphate (PIP) by two distinct enzymes, PI and PIP kinases, which we have recently characterized in human PMN (15, 16). Although the hydrolysis of PIP$_2$ by phospholipase C has been well studied, little is known of the synthesis and thus regeneration of this important phospholipid in leukocytes.

It has been demonstrated that incubation of intact PMN with the chemoattractant, formyl-methionyl-leucyl-phenylalanine (FMLP) results in a rapid decrease in PIP$_2$, followed by a slower increase in cellular levels of this phospholipid (4, 17). Similar observations have been noted in other receptor systems such as in the action of epidermal growth factor on A431 tumor cells, except that increases in PIP$_2$ are noted following the initial receptor-mediated hydrolysis of PIP$_2$ (18). Epidermal growth factor was subsequently shown to directly activate the first enzyme in the sequence, PI kinase (18). More recently, Auger et al. (19) have shown that anti-phosphotyrosine immunoprecipitates from platelet-derived growth factor-stimulated smooth muscle cells contain lipid kinases which phosphorylate phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate to produce the novel phosphoinositides, phosphatidylinositol 3,4-diphosphate and phosphatidylinositol trisphosphate. These kinase activities are absent in immunoprecipitates of resting cells. Based on these observations, we sought to determine whether chemoattractants altered the activity of PI and/or PIP kinases in human PMN. We report here that chemoattractants cause an activation of PIP kinase, but not PI kinase. The activation of PIP kinase appears to be due to an increase in the $V_{\text{max}}$ of the enzyme.

**EXPERIMENTAL PROCEDURES**

Chemicals—PI, PIP, PIP$_2$, Trizma base, ATP, Triton X-100, FMLP, leukotriene B$_4$ (LTB$_4$), A23187, phorbol myristate acetate (PMA), N-formyl-norleucyl-leucyl-phenylalanine (FMLP, Leu-Leu-Phe-Nle-Tyr-Lys), N-formyl-methionyl-methionyl methyl ester (Met-Met), N-formyl-methionyl-phenoxyalanine (Met-Phe), phenylmethylsulfonyl fluoride, leupeptin, EGTA, benzamidine, cycloheximide, cyclohexilalanin B, and superoxide dismutase were from Sigma. Hanks’ balanced salt solution was from Gibco. $[^3]$P[ATP (3000 Ci/mmol), [2,5,6,2H]ATP, and inositol [2-$^3$H]PIP$_2$ were obtained from Du Pont-New England Nuclear.

**Cell Preparation**—Peripheral blood human PMN were isolated by sedimentation in 3% dextran (Pharmacia LKB Biotechnology Inc.), 0.9% NaCl, followed by centrifugation on Lymphoprep (Oregon Tek-
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RESULTS

Effects of Chemoattractants on PI and PIP Kinases in Human PMN—To determine whether the chemoattractant, FMLP, altered the activity of PI and PIP kinases in human PMN, intact cells were incubated at 37 °C for times ranging from 30 s to 10 min with HBSS alone or containing 50 nM FMLP. Fig. 1 shows the effects of FMLP treatment on both PIP kinase (Fig. 1A) and PI kinase (Fig. 1B) activities. PIP kinase activity (Fig. 1A) was increased as early as 30 s following addition of FMLP to the cells, and maximal stimulation of 97% occurred at 3 min (p < 0.025). The increase in PIP kinase activity was noted during the entire 10 min incubation time. In contrast, PI kinase activity (Fig. 1B) was not altered by incubation of the cells with FMLP.

Fig. 2 shows the dose response effect of FMLP for stimulation of PMN PIP kinase. Incubation of PMN at 37 °C for 3 min with concentrations of FMLP ranging from 1 nM to 0.1 μM caused a dose-dependent stimulation of PIP kinase with a maximal increase in activity noted at a concentration of 50 nM, a dose which produces maximal chemotactic activity in a modified Boyden chamber chemotaxis assay (20). Of note, PI kinase activity was not altered at any concentration of FMLP tested.

To determine whether other formyl peptide chemoattractants were capable of stimulating PIP kinase activity in human PMN, cells were incubated with various concentrations of FMLP, Nle-Leu-Phe-Nle-Tyr-Lys, fMet-Met-Met, and fMet-Phe for 3 min at 37 °C prior to isolation of particulate membrane fractions which were then assayed for PIP kinase activity. Table I shows the concentration of peptide which produced maximal stimulation of PIP kinase activity. The order of potency for stimulation of PIP kinase was Nle-Leu-Phe-Nle-Tyr-Lys > FMLP > fMet-Met-Met > fMet-Phe. This order of peptide potency for stimulating PMN PIP kinase exactly parallels the order of potency of these peptides.
stimulation was dose-dependent, and increased PIP kinase for 3 min at 37 °C, and the membrane fraction isolated as described in "Experimental Procedures." Fig. 3 shows that LTB, increased PIP kinase activity in human PMN. The specific activity = 442 cpm/pmol and 7.5 pg of PIP. Results shown are +

| Cells incubated with | Concentration | Specific activity | HBSS control
|---------------------|---------------|-----------------|----------------|
|                     | nM            | pmol 32PIP2 formed/min/mg protein | %
| HBSS                |                | 28.9 ± 1.6      | 100            |
| /Nle-Leu-Phe-Nle-Tyr-Lys | 10            | 46.6 ± 0.7      | 173            |
| /Met-Leu-Phe         | 50            | 37.9 ± 0.2      | 140            |
| /Met-Met-Met         | 100           | 46.1 ± 1.4      | 171            |
| /Met-Phe             | 1000          | 47.6 ± 4.9      | 177            |

° PMNs were incubated with HBSS alone or the indicated peptide for 3 min at 37 °C, and the membrane fraction isolated as described in the text.

Assays were performed in the presence of 0.1 mM [γ-32P]ATP (specific activity = 442 cpm/pmol) and 7.5 μg of PIP. Results shown are ± S.D. of duplicate samples in a representative experiment performed three times with similar results.

° Percent of HBSS control = (E/C) × 100 where E = PIP kinase specific activity in the presence of chemoattractant and C = the specific activity in the presence of buffer alone.

for inducing functional responses such as chemotaxis, superoxide production, and lysosomal enzyme release in PMN (20, 23).

Experiments were performed to determine whether another chemoattractant, LTB, also altered PIP kinase activity in human PMN. LTB has been shown to bind to a class of cell surface receptors which are distinct from those that bind formyl peptide chemoattractants (24, 25). Human PMN were incubated with HBSS alone or containing concentrations of LTB, ranging from 0.1 nM to 1.0 pM for 3 min at 37 °C, following which the particulate membrane fraction was isolated as described in “Experimental Procedures.” Fig. 3 shows that LTB, increased PIP kinase activity in human PMN. The stimulation was dose-dependent, and increased PIP kinase activity from 47.9 ± 1.1 pmol 32PIP2 formed per min/mg protein in the presence of buffer to 66.7 ± 0.2 pmol of 32PIP2 formed per min/mg protein in the presence of 50 nM LTB,.

This concentration of LTB, has been shown to maximally stimulate the chemotactic response in human PMN (24–26). As with the formyl peptide chemoattractants, no change in PI kinase activity was noted in membranes isolated from LTB-treated PMNs.

**TABLE I**

Effect of N-formylated peptide chemoattractants on PMN PIP kinase activity

**FIG. 2.** Effect of FMLP on PMN PI and PIP kinases: dose response. Intact cells were incubated for 3 min at 37 °C with HBSS alone or containing concentrations of FMLP ranging from 0.1 nM to 0.1 μM. PI (○) and PIP (○) kinase activities were measured in the presence of 0.1 mM [γ-32P]ATP and 50 μg/ml PI or 75 μg/ml PIP, respectively. ○, 32PIP2 formed; ●, 32PIP formed. The experiment was performed 3 times with similar results.

**FIG. 3.** Effect of LTB, on PMN PI and PIP kinases: dose response. Intact cells were incubated with HBSS alone or containing concentrations of LTB, ranging from 0.1 nM to 1.0 pM for 3 min at 37 °C following which PI (○) and PIP (○) kinase activities were measured in the presence of 0.1 mM [γ-32P]ATP and 50 μg/ml PI or 75 μg/ml PIP, respectively. ○, 32PIP2 formed; ●, 32PIP formed. The experiment shown is representative of three performed.

**Kinetic Properties of the Chemoattractant-stimulated PMN PIP Kinase**—Particulate membrane fractions were prepared from human PMN incubated with or without 50 nM FMLP for 3 min at 37 °C and were assayed for PIP kinase activity in the presence of 75 μg/ml PIP and concentrations of ATP ranging from 0.01 to 2 mM ATP (22.1-4420 cpm/pmol). Fig. 4 shows a representative experiment in which PIP kinase activity is increased at all concentrations of ATP tested in membranes isolated from FMLP-treated cells. The inset (Fig. 4) shows Lineweaver-Burk analysis of this representative experiment. The Vmax calculated as the mean ± S.E. of three identical experiments was 14.4 ± 2.6 pmol of 32PIP2 formed per min/mg protein in membranes isolated from HBSS-treated cells and 27.4 ± 4.4 pmol of 32PIP2 formed per min/mg protein in membranes isolated from FMLP-treated cells. There was no significant change noted in the apparent Km for ATP of PIP kinase activity in membranes isolated from FMLP-treated cells (buffer, 0.07 ± 0.01 mM; FMLP, 0.09 ± 0.01 mM, n = 3).

**Effects of Nonchemoattractant Activators of the Human PMN Oxidative Burst on PIP and PI Kinase Activities**—Since chemoattractants cause stimulation of the oxidative burst in human neutrophils manifested by superoxide production and lysosomal enzyme release (27, 28), experiments were performed to determine whether nonchemoattractant activators of PMN were capable of stimulating PIP kinase activity. Fig. 5 shows a comparison of the amounts of superoxide produced by the chemoattractants, FMLP and LTB, as well as the phorbol ester and protein kinase C activator PMA and the calcium ionophore A23187. PMA was the most active stimulator of PMN superoxide production. Maximal stimulation of 3.65 nmol/10⁶ cells/10 min was noted in the presence of 0.1
Reduction of ferricytochrome c was measured as an index of superoxide production. The production of superoxide was calculated by subtracting the amount produced in the absence of superoxide dismutase. Results shown are the difference in the production of superoxide in the presence of the indicated activator minus the amount produced in the presence of buffer (PMMA). No reduction of cytochrome c above a buffer background was noted in cells treated with superoxide dismutase. Results shown are the average of three identical experiments performed.

If the stimulation of PIP kinase by chemoattractants was evaluated, isolated PMN were incubated with concentrations of PMA ranging from 10 nM to 0.5 µM for 3 min at 37 °C, following which the particulate membrane fractions were isolated and assayed for PI and PIP kinase activities. Fig. 6A shows that PMA produced no significant alterations in PI or PIP kinase activities, but inhibited both enzymes by as much as 50% (Fig. 6B) at concentrations which exceeded those that produced maximal stimulation of superoxide anion production. No significant stimulation of either enzyme was noted when the incubation time of 0.5 µM PMA with cells was varied from 30 s to 10 min (data not shown).

Incubation of PMN with concentrations of A23187 ranging from 0.1 to 10 µM for 3 min at 37 °C, similarly, did not stimulate PI or PIP kinase activities, but inhibited both enzymes by as much as 50% (Fig. 6B) at concentrations which exceeded those that produced maximal stimulation of superoxide anion production. No significant stimulation of either enzyme was noted when the incubation time of 0.5 µM PMA with cells was varied from 30 s to 10 min (data not shown).

Effect of Cytochalasin B on Chemoattractant-induced Stimulation of PMN PIP Kinase—The anti-microfilament agent, cytochalasin B, augments the oxidative burst of PMN to soluble stimuli such as chemoattractants (31, 32). To determine whether this agent altered the stimulation of PMN PIP kinase in response to a submaximal stimulation by FMLP, isolated PMN were preincubated with HBSS alone or containing concentrations of cytochalasin B ranging from 0.1 to 5 µM for 10 min at 37 °C prior to the addition of HBSS or 10 nm FMLP. Incubations were terminated after 5 min and the particulate membrane fractions isolated and assayed for PIP kinase activity. Fig. 7 shows that cytochalasin B alone caused a significant (p < 0.005) increase in PIP kinase activity at the highest concentration tested, 5 µM. The presence of calcium has been shown to inhibit both PI and PIP kinases derived from human PMN (31).
however, in the presence of cytochalasin B, there was a dose-dependent augmentation of PIP kinase activity. In the presence of 5 μM cytochalasin B, FMLP increased the enzyme activity to 70.7 ± 2.7 pmol of 32PIP2 formed per min/mg protein, a value which is 3.9-fold that noted in cells incubated with HBSS alone.

**Effects of Exogenous PIP and PIP2 on the Stimulation of PIP Kinase by FMLP—**FMLP treatment of intact neutrophils results in a rapid decrease in both PIP and PIP2 levels with more dramatic effects occurring on the latter phospholipid. After the initial decrease, PIP2 levels are noted to increase after the first minute and PIP levels return to base line. In addition, PIP2 has been shown to be an end product inhibitor of PIP kinase. Given these considerations, it was important to determine whether chemoattractant-induced stimulation of PIP kinase in human neutrophils might be due to resultant increased levels of PIP as substrate or decreased levels of PIP2 as end product inhibitor.

To address these possibilities, intact cells were incubated with HBSS alone, or containing 50 nM FMLP for 3 min at 37 °C following which particulate membrane fractions were prepared and assayed for PIP kinase in the presence of 0.1 mM [γ-32P]ATP and 75 μg/ml PIP, respectively. Results are expressed as percent of Normal = E/C x 100 where E is the specific activity of the enzyme derived from cells incubated in the presence of PMA or A23187 and C is the specific activity of PIP kinase activity derived from cells incubated with HBSS alone. ○, 32PIP formed; ○, 32PIP2 formed. A is representative of five experiments performed; B is representative of three experiments.

The effects of exogenous PIP2 addition were similarly evaluated. Concentrations of PIP2 ranging from 0 to 50 μg/assay (0-500 μg/ml) did not significantly alter the relative stimulation of PIP kinase by FMLP, though the overall enzyme activity in both HBSS- and FMLP-treated membranes was inhibited by concentrations of PIP2 ≥ 10 μg/assay (data not shown).

**Effect of FMLP on the Hydrolysis of ATP—**To determine if the stimulating effects of FMLP on PIP kinase could be explained by protection of ATP from hydrolysis, the rate of ATP degradation was measured in the presence of particulate membrane fractions which were prepared from PMN incubated with HBSS for 3 min at 37 °C with or without 10 nM FMLP. [3H]ATP (0.1 mM, 5 μCi) was incubated with 50 mM Tris, 10 mM MgCl2, pH 7.4, and 7.5 μg of PIP and particulate membrane fractions derived from HBSS- or FMLP-treated cells for 1-5 min at 25 °C. Reactions were stopped by the addition of methanol and rapid centrifugation, and adenosine nucleotides were separated by thin layer chromatography as described under "Experimental Procedures." Fig. 8 shows that there were no significant differences at any time tested in the amount of [3H]ATP remaining in incubations containing membranes from HBSS- or FMLP-treated cells. In addition, the overall degradation of [3H]ATP measured during the 5-min incubation period was minimal under both conditions (96.2 ± 6.5% [3H]ATP remaining in the presence of HBSS membranes; 85.5 ± 7.3% [3H]ATP remaining in the
inhibition of PIP* metabolism, phospholipase C and phospho-
memoesterase activity of particulate membrane fractions from HBSS- or FMLP (50 nM)-treated cells was measured under conditions used to measure PIP kinase activity. Membrane fractions (75 μg of protein) and the formation of inositol phosphates was measured spectrophotometrically and is expressed as the residual counts/min ± S.D. of duplicate samples. Time 0 represents [3H]ATP incubated in the absence of membranes. The results are representative of two experiments performed.

presence of FMLP membranes). The enhancement of PIP kinase by FMLP could therefore not be explained by a decrease in ATP hydrolysis.

**Effects of FMLP on the Hydrolysis of PIP2**—To determine if the stimulatory activity of FMLP in PIP kinase was due to inhibition of PIP metabolism, phospholipase C and phosphomoesterase were measured under conditions used to measure PIP kinase activity. Membrane fractions (75 μg of protein) derived from HBSS or FMLP (50 nM)-treated cells were incubated for 5 min at 25 °C with [3H]ATP (5 μCi, 0.1 mM) and 7.5 μg of PIP for the indicated times at 25 °C. Reactions were stopped and adenosine nucleotides were separated by TLC as described under “Experimental Procedures.” The residual [3H]ATP was measured spectrophotometrically and is expressed as the residual counts/min ± S.D. of duplicate samples. Time 0 represents [3H]ATP incubated in the absence of membranes. The results are representative of two experiments performed.

**Fig. 8. Effects of FMLP on the hydrolysis of [3H]ATP by PMN membranes.** Particulate membrane fractions were isolated from cells which had been treated with HBSS alone (○) or 50 nM FMLP (□) and incubated with [3H]ATP (5 μCi, 0.1 mM) and 7.5 μg of PIP for the indicated times at 25 °C. Reactions were stopped and adenosine nucleotides were separated by TLC as described under “Experimental Procedures.” The residual [3H]ATP was measured spectrophotometrically and is expressed as the residual counts/min ± S.D. of duplicate samples. Time 0 represents [3H]ATP incubated in the absence of membranes. The results are representative of two experiments performed.

Membrane fractions derived from PMN treated with HBSS alone or containing 50 nM FMLP were isolated in 50 mM Tris in the absence of Mg**++.** The isolated membranes were then assayed for PIP kinase in the presence of MgCl2 concentrations ranging from 0.1 to 20 mM, 75 μg/ml PIP, and 0.1 mM [γ-32P]ATP (442 cpm/pmol). The apparent Kₐ values for Mg**++**-induced stimulation of PIP kinase activities isolated from buffer and FMLP-treated cells were identical (0.9 mM). Similarly the Kₐ values for Ca**++**-induced inhibition of basal and FMLP-stimulated PIP kinase activities were the same (2 mM).

Experiments were also performed to determine whether protein synthesis was necessary for chemotactic-stimulated stimulation of PIP kinase. Isolated human PMN were incubated with HBSS alone or with the protein synthesis inhibitor, cycloheximide (100 μg/ml) for 10 min at 37 °C, following which either HBSS or 50 nM FMLP was added for an additional 3 min at 37 °C. The particulate membrane fractions were then isolated and assayed for PIP kinase activity. In the presence of HBSS alone, PIP kinase activity was 17.0 ± 0.5 pmol of 32P incorporated per min/mg protein, and in the presence of 50 nM FMLP, the activity was 31.3 ± 1.5 pmol of 32P incorporated per min/mg protein. When cells were incubated in the presence of 50 nM FMLP and 100 μg/ml cycloheximide, PIP kinase activity in the isolated membranes was 32.3 ± 0.15 pmol/min/mg protein, a value not significantly different from that observed in the presence of FMLP alone (p > 0.2). Protein synthesis as measured by the incorporation of [3H]leucine into acid-precipitable radioactivity in human PMN preparations was inhibited by 96.3% in the presence of 100 μg/ml cycloheximide (data not shown). Cycloheximide itself had no effect on basal PIP kinase activity.

**DISCUSSION**

Many hormones and neuropeptides as well as chemotactants institute receptor-mediated transmembrane signaling via activation of phospholipase C with subsequent degra-
dation of PIP₂ and generation of the second messengers inositol 1,4,5-trisphosphate and diacylglycerol (4, 6, 8, 10).

Many of these receptor systems including those for N-formyl peptide chemoattractants require intermediate coupling with guanine nucleotide regulatory proteins (6, 33–35). The phosphorylated derivative of PI, PIP₂, is the necessary substrate for phospholipase C in this reaction but is present in very small amounts within eukaryotic cells, suggesting that its synthesis is under stringent regulation. We report here that the synthesis of PIP₂ is directly influenced by chemotactic-receptor occupancy in human neutrophils. The incubation of intact cells with N-formylated chemoattractants or leukotriene B₄ caused a rapid (within 30 s) stimulation of PIP kinase with a maximal effect occurring at 3 min at 37 °C. The increase in the enzyme activity was dose-dependent over the range of concentrations of these substances which have been shown to stimulate chemotaxis maximally in Boyden chamber assays in vitro (20, 26, 27). No stimulation of PI kinase activity was noted in identical membrane preparations derived from chemoattractant-stimulated cells, indicating that these agents do not globally and nonspecifically alter enzymes involved in phospholipid synthesis.

The chemoattractant-induced increase in PIP kinase activity was not a nonspecific consequence of superoxide anion generation in PMN, since cellular activators such as PMA and A23187 did not significantly increase PIP kinase activity at concentrations which stimulated superoxide production.
much more effectively than the chemoattractants, FMLP and LTB4 (Fig. 5). In addition, a concentration of superoxide dismutase (50 μg/ml) which completely inhibited detection of superoxide production by various cellular activators did not interfere with the ability of FMLP to stimulate PMN PIP kinase activity (data not shown).

Interestingly, cytochalasin B, which binds to actin and disrupts microfilaments, markedly increased the ability of FMLP to stimulate PIP kinase. We have previously demonstrated that antimicrofilament agents increase the extractability of PMN PIP kinase in nonionic detergents (36). The explanation for the augmentation of FMLP-induced stimulation of PIP kinase by cytochalasin B is unknown at this time; however, it has been repeatedly demonstrated that antimicrofilament agents greatly increase receptor-mediated stimulation of the oxidative burst by chemoattractants in leukocytes (31, 32).

Chemoattractant-stimulated PIP kinase activity was characterized by as much as a two-fold increase in the V_{max} of the enzyme without a significant effect on the apparent K_{m} for ATP determined in the presence of saturating concentrations of PIP. The increased production of [32P]PIP2 by FMLP could not be explained by an inhibition of ATP hydrolysis nor by inhibition of PIP2 phosphomonoester activity or phospholipase C activity.

The mechanism of the increase in the V_{max} of PIP kinase by chemoattractants is unknown at this time but does not appear to be due to changes in the Mg^{2+} requirements of the enzyme nor in its sensitivity to inhibition by calcium. In addition, new protein synthesis did not appear to be required for the stimulation of PIP kinase by chemoattractants. Treatment of cells with chemoattractants may result in either removal of a tonic inhibitor of PIP kinase activity or in generation or stimulation of an activator of the enzyme. We cannot explain the stimulation of the enzyme by FMLP, however, on the basis of increases in PIP levels or decreases in PIP2, a known inhibitor of PIP kinase in human PMN, since the addition of large excesses of both these phospholipids did not alter the level of stimulation of PIP kinase in membranes isolated from FMLP-treated cells. Since guanine nucleotide regulatory proteins are required for coupling of chemoattractant receptor-mediated signals in leukocytes through phospholipase C activation (6), it is possible that these membrane elements also mediate the stimulation of PIP kinase. Indeed, Smith and Chang (37) have shown that non-hydrolyzable analogues of GTP directly stimulate rat brain PIP kinase activity. We are actively investigating this association at present.

The chemoattractant-stimulated PIP kinase activity may be similar to that observed in immunoprecipitates of platelet-derived growth factor-stimulated smooth muscle cells (19). Our method of separation of the phosphoinositides does not distinguish between isomers of PIP2, such as phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 4,5-bisphosphate. Further studies are needed to evaluate this potential association. Traynor-Kaplan et al. (38) have demonstrated the appearance of an inositol tetrakisphosphate-containing phospholipid (phosphatidylinositol trisphosphate) in intact human neutrophils treated with chemoattractants. Increased levels of phosphatidylinositol trisphosphate have also been demonstrated in platelet-derived growth factor-stimulated smooth muscle cells (19). From the present data, we cannot eliminate the possibility that a novel enzyme, PIP2 kinase, is also stimulated in human neutrophils by chemoattractants. We are actively investigating this at present.

Whatever the mechanism, these findings demonstrate direct, chemoattractant-mediated stimulation of PIP kinase, the enzyme which forms the reservoir for second messenger substances. PIP2. Increased synthesis of PIP2 by chemoattractant-stimulated PIP kinase may serve to replenish this important phospholipid in the membrane following its hydrolysis by phospholipase C.

Acknowledgment—We would like to thank Karen Graham for her excellent assistance in the preparation of this manuscript.

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