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Secretagogue-induced phosphoinositide metabolism in human leucocytes

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The relationship between receptor binding of the formylated peptide chemoattractant formylmethionylleucylphenylalanine (fMet-Leu-Phe), lysosomal enzyme secretion and metabolism of membrane phospholipids was evaluated in both human polymorphonuclear leucocytes (PMN) and the dimethyl sulphoxide (Me_2SO)-stimulated human myelomonocytic HL-60 leukaemic cell line. In both cell types, exposure to fMet-Leu-Phe (100nM) induced rapid lysosomal enzyme secretion (maximal release <30s) and marked changes in the ^{32}P-labelling of the inositol lipids phosphatidylinositol (PtdIns), phosphatidylinositol 4-phosphate (PtdIns4*P*), phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] as well as phosphatidic acid (PtdA). Specifically, levels of [^{32}P]PtdIns and [^{32}P]PtdIns(4,5)P_2 decreased rapidly (peak decrease at 10–15s), with a subsequent increase at 30s and later. PtdIns4*P* and PtdA showed only an increase. In Me_2SO-differentiated HL-60 cells prelabelled with [^3H]inositol for 20h, fMet-Leu-Phe caused a net increase in the cellular content of [^3H]inositol phosphates, including a rapid increase in [^3H]inositol 1,4,5-trisphosphate, suggesting that PtdIns(4,5)P_2 breakdown occurs by a phospholipase C mechanism. Both lysosomal enzyme secretion and changes in phospholipid metabolism occur over the same agonist concentration range with a similar time course. Binding of [^3H]fMet-Leu-Phe, although occurring over the same concentration range, exhibited markedly slower kinetics. Although depletion of extracellular Ca^{2+} had no effect on ligand-induced polyphosphoinositide turnover, PtdIns turnover, PtdA labelling and lysosomal enzyme secretion were severely curtailed. These studies demonstrate a receptor-mediated enhancement of phospholipid turnover that correlates with a specific biological response to fMet-Leu-Phe. Further, the results are consistent with the idea that phospholipase C-mediated degradation of PtdIns(4,5)P_2, which results in the formation of inositol trisphosphate, is an early step in the stimulus–secretion coupling pathway of the neutrophil. The lack of correlation between these two responses and the equilibrium-binding condition suggests that either these parameters are responsive to the rate of ligand–receptor interaction or only fractional occupation is required for a full biological response.

Abbreviations used: fMet-Leu-Phe, formylmethionylleucylphenylalanine; PMN, polymorphonuclear leucocytes; PtdIns, phosphatidylinositol; PtdIns4*P*, phosphatidylinositol 4-phosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PtdA, phosphatidic acid; Ins*P*, inositol phosphate; InsP_2, inositol bisphosphate; InsP_3, inositol trisphosphate. Me_2SO, dimethyl sulphoxide; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; DNAase I, deoxyribonuclease I.

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The interaction of the tripeptide fMet-Leu-Phe with specific receptor sites on leucocytes induces a number of functional responses, including directed cell mobility, secretion of granular enzymes and enhancement of respiratory-burst activity (Becker, 1979; Schiffmann, 1982). These responses are associated with several biochemical perturbations within the cell, including a redistribution of cations (Sha'afi & Naccache, 1981), phospholipid (Hirata *et al.*, 1979) and protein (O'Dea *et al.*, 1978) methylation reactions, protein phosphorylation reactions (Schneider *et al.*, 1981; Andrews &

Babior, 1983), enhanced turnover of arachidonic acid in membrane phospholipids (reviewed by Becker *et al.*, 1981) and the enhanced metabolism of cellular inositol phosphatides (Cockcroft, 1982; Serhan *et al.*, 1982; Volpi *et al.*, 1983).

The suggestion of Michell (1975) that the phosphodiesteratic hydrolysis of PtdIns, or, more recently, of the polyphosphoinositides PtdIns4*P* and PtdIns(4,5)P_2 (Kirk *et al.*, 1981), may be early events in transduction processes linking receptor occupation with functional responses has been evaluated in numerous cell systems (Michell, 1982).

The studies reported herein were designed to evaluate the peptide-induced metabolism of the cellular phospholipids PtdIns, PtdIns4*P*, PtdIns(4,5)P_2 and PtdA in human PMN and in Me_2SO-stimulated HL-60 myelomonocytic leukaemia cells. The latter have been shown to possess functional receptors for fMet-Leu-Phe, the biochemical properties of which are indistinguishable from receptors on human PMN (Fontana *et al.*, 1980; R. W. Dougherty, R. A. Carchman & R. J. Freer, unpublished work). The changes induced by fMet-Leu-Phe in the metabolism of these phospholipids were compared with respect to both time and agonist concentration to the specific binding of [^{3}H]fMet-Leu-Phe and to peptide-induced secretion. Finally, the dependencies of both phospholipid metabolism and lysosomal enzyme secretion on the presence of extracellular Ca^{2+} were evaluated.

Materials and methods

Materials

The following chemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.: bovine serum albumin (Cohn fraction V), cytochalasin B, *p*-nitrophenyl-β-D-glucosaminidase, Histopaque (Ficoll/Hypaque), EGTA, Hepes and DNAase I. Me_2SO was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Triton X-100 was a gift from Rohm and Haas, Philadelphia, PA, U.S.A. fMet-Leu-Phe was synthesized as described by Freer *et al.* (1980).

HL-60 cell culture

HL-60 myelomonocytic leukaemia cells were obtained from Dr. Robert Gallo (National Cancer Institute, Bethesda, MD, U.S.A.) and grown in tissue-culture flasks (Corning) in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated (56°C, 30min) foetal-bovine serum and 2mM-glutamine (Flow Laboratories, McClean, VA, U.S.A.). Cellular differentiation was achieved by culturing cells for 7 days in the presence of Me_2SO (1.3%, v/v). Before assay, cells were collected by centrifugation and washed twice with the following incubation buffer (concns. in mM): NaCl (140.0)/KCl (5.0)/$NaHCO_3$ (2.8)/$CaCl_2$ (1.5)/$MgCl_2$ (1.0)/$MgSO_4$ (0.06)/glucose (5.6)/Hepes (15.0), pH7.2. Cells were kept on ice until used.

PMN isolation

A portion (40ml) of heparinized (10U/ml) whole blood, obtained with consent from healthy donors (Virginia Commonwealth University Clinical Research Center for Periodontal Disease) was mixed with 10ml of a 6% (w/v) dextran T-500 (Pharmacia, Piscataway, NJ, U.S.A.) solution in 0.9% NaCl. Erythrocytes were allowed to sediment for 30min in an environmentally controlled incubator [37°C, 98% humidity, CO_2/air (1:19)]. The leucocyte-enriched upper layer was removed and diluted 1:1 with 0.9% NaCl. A 25ml portion of this cell suspension was layered over 12.5ml of Histopaque and centrifuged (650***g***, 30min, 20°C). The supernatant was decanted, and contaminating red cells were removed by brief (30s) hypo-osmotic shock in 30ml of deionized water. Iso-osmoticity was restored by addition of 10ml of a 3.6% (w/v) NaCl solution. The suspended granulocytes (>97% PMN, >98% viable) were pelleted by centrifugation, washed twice with incubation buffer (see above) and kept on ice until used.

Phospholipid labelling and analysis

[^{32}*P*]P_i. Cells (25×10^6/ml) were suspended in thermally equilibrated (37°C) incubation buffer supplemented with DNAase I (50units/ml; to prevent cell aggregation) and bovine serum albumin (0.025%). Carrier-free [^{32}P]P_i (New England Nuclear Corp., Boston, MA, U.S.A.) was added to a final concentration of 75μCi/ml of cells. Preliminary studies indicated that the presence of bovine serum albumin in the incubation mixture was an absolute requirement for cells to incorporate [^{32}P]P_i into ATP pools. After a 2h labelling period, cells were centrifuged and resuspended in an equivalent volume of non-radioactive buffer containing cytochalasin B (5μg/ml of cells) in the absence or presence of 1.8mM-EGTA (Ca^{2+}-depletion studies). After a further 5min incubation, cells were stimulated with fMet-Leu-Phe and 500μl samples were removed at timed intervals. The reactions were stopped by quenching cell suspensions directly into 6vol. of chloroform/methanol/6M-HCl (500:1000:3, by vol.). Lipids were extracted at room temperature for 30min, after which time 1ml each of chloroform and 2M-KCl were added. The sample was mixed and then briefly centrifuged (1000***g***, 2min) to resolve the two phases. The lower (organic) phase was removed and washed twice with equal volumes of methanol/1M-HCl (1:1, v/v). The washed organic

phase was dried under N_2, redissolved in 100μl of chloroform/methanol (9:1, v/v), and 50μl applied to separate heat-activated (110°C, 60min, cooled in a desiccator) silica-gel G t.l.c. plates (Merck). One plate was developed in the solvent system chloroform/methanol/acetic acid/water (40:10:10:1, by vol.) to a point approx. 0.2cm from the top of the 10cm plate. After brief drying under forced air, the plate was developed again in the same dimension to a point 1.2cm from the top of the plate. This method afforded good separation of phosphatidylcholine, PtdIns, phosphatidylserine, phosphatidylethanolamine, PtdA and the neutral-lipid fractions. The other plate was developed in chloroform/methanol/1.5M-NH_3/water (90:90:7:22, by vol.) to resolve PtdIns4*P* and PtdIns(4,5)P_2. Lipids were detected by exposure to I_2 vapour, and radioactivity was located by autoradiography on Kodak XAR-5 film. Areas of silica gel corresponding to PtdIns, PtdA, PtdIns4*P*, and PtdIns(4,5)P_2 were scraped into scintillation vials (4ml capacity) with 3ml of Budget-Solve (Research Products International Corp., Mt. Prospect, IL, U.S.A.) and the radioactivity quantified by liquid-scintillation spectrometry. All determinations were done in triplicate. Average ($\pm$S.E.M.; $n = 41$) incorporations of label into each lipid in HL-60 cells were (c.p.m.): PtdIns (30243$\pm$1444), PtdA (25379$\pm$1222), PtdIns4*P* (40139$\pm$1777) and PtdIns(4,5)P_2 (67150$\pm$3552).

[^{3}*H*]*Inositol phosphates*. The accumulation of water-soluble [^{3}H]inositol phosphates was determined as described by Downes & Michell (1981) as modified by Berridge *et al.* (1983). In preliminary experiments (not shown) rabbit neutrophils or HL-60 cells were incubated for up to 3h with 2.0μCi of [^{3}H]inositol/ml. However, insufficient radioactivity was incorporated to permit detection of inositol phosphates. When HL-60 cells were incubated for 20h in Eagle's medium containing 2.0μCi of [^{3}H]inositol/ml, both basal and stimulated levels of [^{3}H]inositol phosphates could be readily measured. Thus data for inositol phosphate formation are presented only for the HL-60 cells. All determinations were done in triplicate.

Lysosomal-enzyme secretion

The secretory response of HL-60 cells and PMN was assayed by quantifying the exocytotic release of the lysosomal marker *N*-acetyl-β-glucosaminidase (EC 3.2.1.53). Cells (25×10^6/ml) were resuspended in incubation buffer at 37°C containing DNAase I and cytochalasin B (5μg/ml of cells). After a 5min incubation various concentrations of fMet-Leu-Phe were added. After 5min, cells were separated from the medium by brief centrifugation (10s at maximum speed; Beckman model B Microfuge). A portion (0.1ml) of the cell-free supernatant was incubated at 37°C for 30min with the following enzyme substrate solution: 2.3mM-*p*-nitrophenyl β-D-glucosaminide/1% Triton X-100/0.01% bovine serum albumin in 50mM-citric acid buffer, pH4.5. The reaction was stopped by the addition of 0.5vol. of 25% (w/v) trichloroacetic acid solution (4°C). The samples were centrifuged (10s at maximum speed; Beckman model B Microfuge), a portion of the supernatant was mixed with 1.5 vol. of 1.5M-NH_3 (pH10.7) and the A_{410} of *p*-nitrophenol measured on a Beckman model 25 u.v./visible spectrophotometer. Cell pellets were solubilized in 100μl of a 0.5% Triton X-100 solution and processed for enzyme activity as described above. The total enzyme activity in the cell suspension was calculated by summing the absorbance units for the released enzyme and that of the lysed cell pellet. Results are amounts of enzyme released expressed as a percentage of total enzyme activity. Basal enzyme release was determined in parallel vehicle-only controls and was subtracted from the stimulated-release values.

The rate of enzyme release from HL-60 cells was evaluated by quenching samples of fMet-Leu-Phe stimulated cell suspensions into 2 vol. of ice-cold incubation buffer to arrest the secretory process. Enzyme activity was determined and expressed as detailed above.

Binding

The binding of [^{3}H]fMet-Leu-Phe (New England Nuclear Corp., Boston, MA, U.S.A.; 47.6Ci/mmol was evaluated as follows. Thermally equilibrated (37°C) suspensions of HL-60 cells were added to buffer containing 100nM-[^{3}H]fMet-Leu-Phe in the absence (total binding) or the presence (non-specific binding) of 100μM-non-labelled fMet-Leu-Phe. At various times thereafter, separation of cell-bound from free ligand was accomplished by rapid filtration of cell suspensions on glass-fibre filters (Whatman GF/C) and extensive washing with ice-cold buffer. Air-dried filters were transferred to scintillation vials, 10ml of Budget-Solve was added and the radioactivity quantified by liquid-scintillation spectroscopy. Dissociation reactions were initiated by the addition of 100μM non-labelled fMet-Leu-Phe to binding reactions in progress. Total and non-specific binding was quantified as described above. Specific binding was calculated as the difference between total and non-specific binding and generally represented 90% of the cell-associated ligand.

Results

The inositol phospholipids of both PMN and HL-60 cells incubated with [^{32}P]P_i became labelled

with nearly identical time courses (not shown). Phosphatidate, PtdIns4*P* and PtdIns(4,5)P_2 appeared to reach a steady-state labelling condition by 60min. A slight but steady increase in the labelling of PtdIns4*P* and PtdIns(4,5)P_2 was seen thereafter. The monophosphate ester groups of these lipids exchange rapidly in the cell and their labelling is probably a reflection of the specific radioactivity of intracellular [^{32}P]ATP pools. The labelling of diester phosphate groups in the absence of stimulation appears to be much slower, accounting for the slight enhancement of the labelling of PtdIns4*P* and PtdIns(4,5)P_2 after the first 60min of incubation and for the linear profile of the labelling of PtdIns up to 4h. After 2h of labelling the specific radioactivity of the polyphosphoinositides and PtdA pools would not be expected to change significantly over the duration of the lipid-turnover studies (90s). Therefore these studies were initiated after a 2h labelling period.

^{32}P *turnover*

Stimulation of HL-60 cells with fMet-Leu-Phe (100nM) elicits rapid changes in the activity of each lipid investigated (Fig. 1). Within 10s of agonist stimulation the levels of [^{32}P]PtdIns and

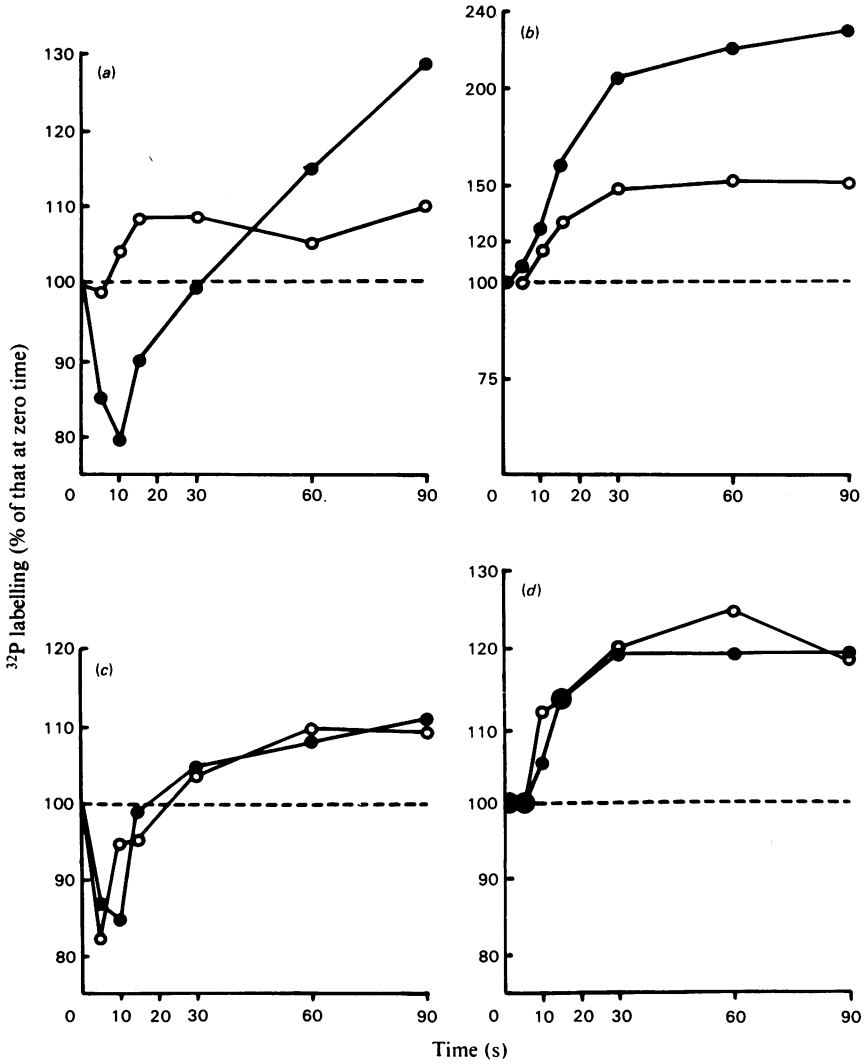

Fig. 1. *Effect of fMet-Leu-Phe on the time course of* [^{32}P]P_i *labelling of PtdIns (a), PtdA (b), PtdIns(4,5)*P_2 *(c) and PtdIns4P (d) in HL-60 cells*

Identical protocols were carried out in the presence (●) and absence (○) of Ca^{2+}. Results are expressed as percentages of the [^{32}P]P_i content in each phospholipid after prelabelling as described in the Materials and methods section. The reaction was initiated by exposure to 100nM-fMet-Leu-Phe at 37°C. Each point represents the average result for at least five experiments performed in triplicate.

[^{32}P]PtdIns(4,5)P_2 showed maximal decrease followed by a recovery in radioactivity that exceeded baseline at later time points. There was no decline in [^{32}P]PtdIns4P at any time, but enhanced labelling of the lipid was evident after a slight lag (5s). Enhanced labelling of PtdA was evident within 5s of receptor stimulation and exceeded twice the control value by 30s. The labelling phases of PtdIns4P, PtdIns(4,5)P_2 and PtdA were complete within 30–60s of stimulation, whereas enhanced PtdIns labelling continued throughout the experimental periods. Qualitatively similar results were obtained with freshly isolated human PMN (Fig. 2). Labelled PtdIns and PtdIns(4,5)P_2 decreased rapidly after stimulation with fMet-Leu-Phe (100nM), subsequently recovered, and either exceeded ([^{32}P]PtdIns) or approached {[^{32}P]PtdIns(4,5)P_2} basal levels by 60s. As with HL-60 cells, only enhanced labelling of PtdIns4P and PtdA were noted.

The magnitude of the decreases in [^{32}P]PtdIns and [^{32}P]PtdIns(4,5)P_2, the enhancement in [^{32}P]PtdA and the secretion of *N*-acetyl-β-glucosaminidase are all dependent on the concentration of agonist. The concentration–response relationships of the net breakdown of [^{32}P]PtdIns and secretion are shown in Fig. 3. Since the net breakdown of [^{32}P]PtdIns is actually a balance between hydrolysis and subsequent resynthesis, the extent of the response shown must be considered a minimum estimate. In order to minimize the complication of data interpretation due to presumed resynthesis, lipid decrease at the 5s time point only is presented. The ED_{50} values (concentrations necessary to produce 50% maximum response) for fMet-Leu-Phe-OH-elicited decreases in PtdIns (40nM) and secretion (35nM), estimated from concentration–response curves, are indistinguishable. Similar results were obtained for the decrease in PtdIns(4,5)P_2 (results not shown).

Alterations in the levels of ^{32}P-labelled phospholipids can be accomplished by any of a variety of enzymes including phosphomonoesterases, phosphodiesterases or acyl hydrolases. In order to gain more insight into the enzymic mechanisms involved in the observed lipid changes, levels of water-soluble inositol phosphates, which would be expected to accumulate due to the action of a phospholipase C on the inositol phosphatides, were examined. In HL-60 cells prelabelled with [^{3}H]inositol, fMet-Leu-Phe induced a rapid accumulation of [^{3}H]InsP_3 and [^{3}H]InsP_2 (Fig. 4) with a more gradual, essentially linear, increase in [^{3}H]Ins$P$ (Fig. 5). The accumulation of [^{3}H]InsP_3, presumably due to hydrolysis of [^{3}H]PtdIns(4,5)P_2, was nearly maximal at the first time point examined (5s), whereas accumulation of [^{3}H]InsP_2 continued up to 30s.

A further requirement for receptor mediation of functional response is that a reasonable temporal sequence relating receptor occupation to that response should be demonstrable. Therefore we have compared the kinetics of the specific binding of [^{3}H]fMet-Leu-Phe with the kinetics of phospholipid breakdown and lysosomal enzyme secretion. The results obtained with [^{3}H]InsP_3 are shown in Fig. 6. Each parameter was measured in separate experiments under similar conditions. It is obvious that both lysosomal enzyme secretion and InsP_3 accumulation proceed rapidly in response to peptide stimulation, with the former being complete within 30s. Formation of InsP_3 is temporally coincident with, or slightly precedes, lysosomal

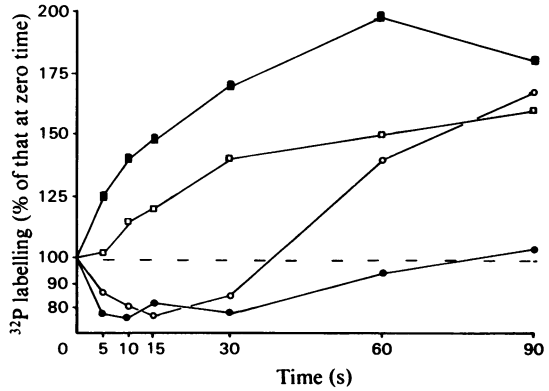

Fig. 2. *Effect of fMet-Leu-Phe on the time course of* [^{32}P]P_i *labelling of PtdIns* (●), *PtdA* (■), *PtdIns(4,5)*P_2 (○) *and PtdIns4P* (□) *in human PMN*
Conditions were as outlined in Fig. 1.

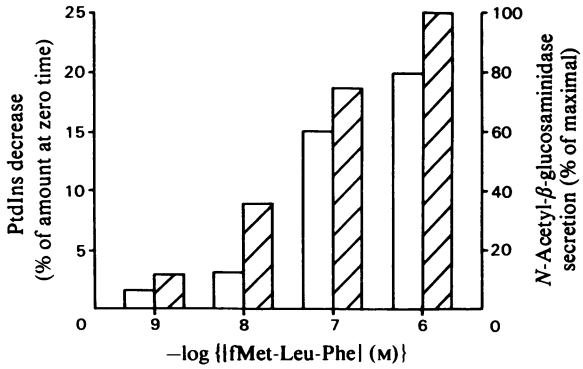

Fig. 3. *Concentration-dependency of fMet-Leu-Phe-induced PtdIns breakdown* (□) *and lysosomal enzyme secretion* (▨) *in HL-60 cells*
PtdIns breakdown was measured 5s after exposure to fMet-Leu-Phe. Lysosomal enzyme secretion was measured after the release reaction was complete. Results are averages for at least five experiments performed in triplicate.

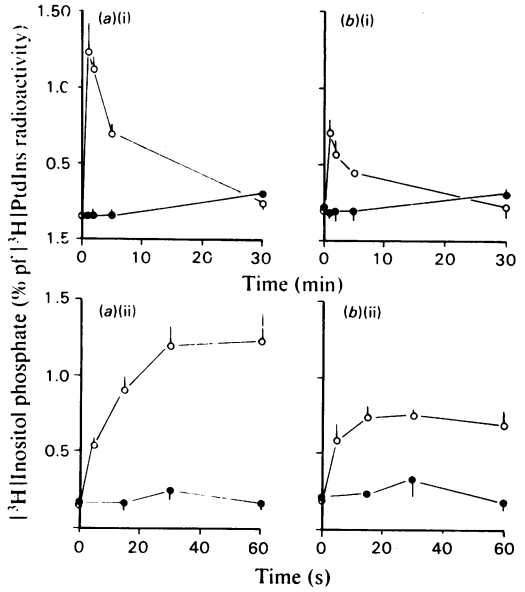

Fig. 4. *Formation of* [^3H]*InsP*$_2$ (*a*) *and* [^3H]*InsP*$_3$ (*b*) *in HL-60 cells*

●, Control cells; ○, cells stimulated at zero time with 100nM-fMet-Leu-Phe. Responses are shown on a time scale of 0–30min (i) and 0–60s (ii). Results here, and in Fig. 5, are expressed as percentages of radioactivity associated with PtdIns, which averaged 69982±3778c.p.m./mg of protein.

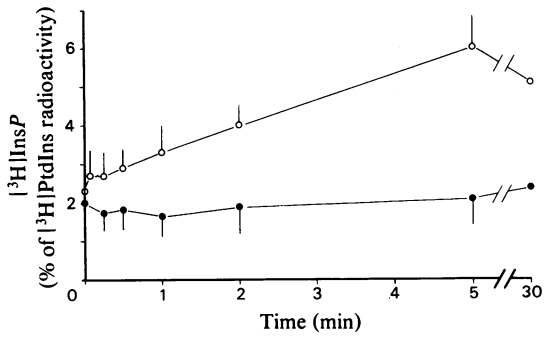

Fig. 5. *Formation of* [^3H]*InsP in HL-60 cells*

●, Control cells; ○, cells stimulated at zero time with 100nM-fMet-Leu-Phe. Results are from the same experiments summarized in Fig. 4.

enzyme secretion. Interestingly, the receptor binding exhibits apparently slower kinetics, with binding still increasing at a time when the biological response and the InsP_3 formation are complete. Similar results have been reported by Sklar *et al.* (1982) and Radin *et al.* (1982). It should be noted, however, that the binding kinetics are complicated by the time-dependent decrease in displaceable specific binding that occurs at 37°C (Fig. 6). At 30s all specifically bound ligand is displaceable, whereas at 90s, 25% of the ligand cannot be displaced, presumably owing to receptor down-regulation, internalization of receptors or both.

In rabbit neutrophils fMet-Leu-Phe-induced alterations in PtdIns and PtdA are dependent on the presence of extracellular Ca^{2+} (Cockroft *et al.*, 1980; Volpi *et al.*, 1983). We have confirmed these results in human PMN (however, see Serhan *et al.*, 1982) and extended this to the HL-60 cells. In addition we have measured stimulated changes in PtdIns4*P* and PtdIns(4,5)P_2 in both cell types. The results for HL-60 cells are shown in Fig. 1. There is a marked attenuation of PtdA labelling and of both breakdown and resynthesis of PtdIns. There is a modest delay (5s) in the onset of PtdA labelling that is not seen in the presence of Ca^{2+}. However, there was no detectable effect on PtdIns(4,5)P_2 breakdown and resynthesis or on the enhanced labelling of PtdIns4*P*. Under these conditions lysosomal enzyme secretion is completely blocked.

Discussion

These studies confirm previous reports indicating that fMet-Leu-Phe stimulates inositol phosphatide metabolism in PMN (Cockcroft *et al.*, 1980; Serhan *et al.*, 1982; Volpi *et al.*, 1983). The analysis reported here strongly suggests that the lipid hydrolysis is mediated by phospholipase(s) of the 'C' type. The evidence for this conclusion is twofold: firstly, the rapidity of the formation of [^{32}P]PtdA suggests that a quantity of diacylglycerol had been made available to a diacylglycerol kinase within seconds of receptor occupation. Logical candidates for sources of this diacylglycerol are the inositol phosphatides, which show temporally relevant decreases in amounts. Secondly, the accumulation of water-soluble inositol phosphates, the other expected products of a phospholipase C-mediated reaction, occurs coincident with the [^{32}P]inositol lipid disappearances and the accumulation of [^{32}P]PtdA. The differential time course of the appearance of the individual [^{3}H]-inositol phosphates suggests that the phosphodiesteratic hydrolysis of PtdIns(4,5)P_2 (and perhaps of PtdIns4*P*), rather than of PtdIns, is the event most proximal to receptor occupation. The rapid decrease in [^{32}P]PtdIns observed on stimulation apparently results from mechanisms not involving phospholipase C, and the possibilities of phospholipase A_2 or phospholipase D mechanisms should be considered. Although the possibility of a delayed, gradual phosphodiesteratic hydrolysis of

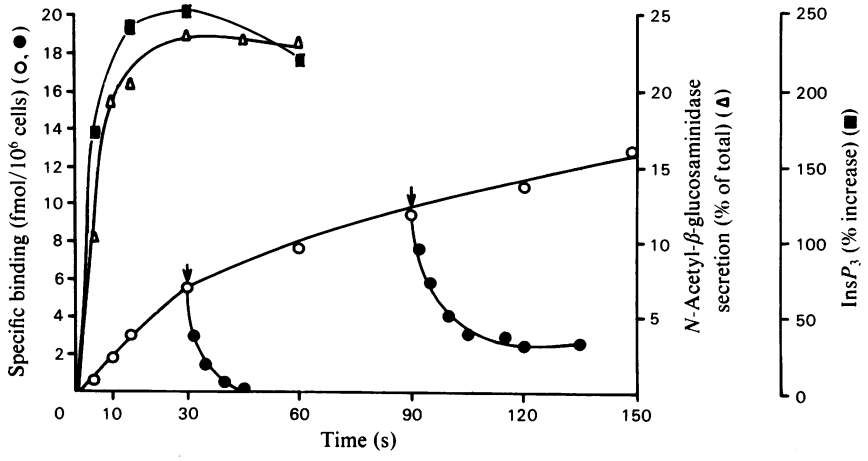

Fig. 6. *Kinetics of lysosomal enzyme secretion* (△) *and InsP_3 formation* (■) *induced by fMet-Leu-Phe* (*100 nM*), *and specific binding of* [^{3}H]*fMet-Leu-Phe* (○)

Displaceable binding of [^{3}H]fMet-Leu-Phe (●) was determined by addition of 100 μM-fMet-Leu-Phe at times after initiation of the binding reaction as indicated by the arrows.

PtdIns cannot be ruled out, we feel that the accumulation of [^{3}H]Ins*lP* results primarily from the stepwise degradation of inositol bis- and tris-phosphates in aqueous cellular compartments. Similar conclusions were reached when other cell systems were studied (Berridge, 1983; Martin, 1983; Aub & Putney, 1984).

The physiological significance of this enhanced inositol phospholipid degradation in 'stimulus–response' coupling processes remains unknown. A feature generally shared by systems displaying this inositol lipid response after receptor occupation is an accompanying elevation in cytosolic Ca^{2+}, suggesting that the metabolism of the inositol phospholipids may contribute in some way to the Ca^{2+} mobilization (Michell, 1975; Michell *et al.*, 1981). Implicit in this argument is the requirement for the primary step in the sequence leading to Ca^{2+} mobilization to be, itself, independent of Ca^{2+} mobilization. These studies have shown the Ca^{2+}-independence of PtdIns(4,5)P_2 hydrolysis. However, breakdown of PtdIns, and to some extent generation of [^{32}P]PtdA, appear to be Ca^{2+}-dependent reactions (the present results; Cockcroft *et al.*, 1980).

Observation of a biochemical event in response to fMet-Leu-Phe does not, in itself, imply a cause-and-effect relationship. Therefore the rate of changes in phospholipid metabolism was compared with the rates of lysosomal enzyme secretion and receptor binding of fMet-Leu-Phe. Within the limitations of the methodologies employed, there was good temporal agreement between all three parameters. This, coupled with the similar concentration-dependency for each, is highly supportive of the notion that both inositol lipid breakdown and lysosomal enzyme secretion are initiated by the same ligand–receptor interaction. Further, the rapidity of InsP_3 formation suggests that formation of this molecule could precede, and thus trigger, the events involved in lysosomal enzyme secretion. A recent report has shown that InsP_3 can, in submicromolar concentrations, release Ca^{2+} from ATP-dependent intracellular pools in pancreatic acinar cells (Streb *et al.*, 1983). A similar action for InsP_3 in the neutrophil has not been directly demonstrated. However, it is generally agreed that Ca^{2+} mobilization in the neutrophil involves release from internal pools. It is therefore tempting to speculate that, in neutrophils and HL-60 cells, InsP_3 may serve as the second messenger, coupling surface membrane receptor activation to internal Ca^{2+} release, as previously suggested for other cell types (Berridge, 1983; Streb *et al.*, 1983).

In summary, these results indicate that phospholipid responses of human PMN and HL-60 cells are similar and demonstrate that the Me_2SO-differentiated HL-60 cell is a good model for studying receptors and secretory mechanisms in the PMN. In addition, the data extend previous findings on the role of phosphoinositide metabolism by demonstrating, for the first time, Ca^{2+}-independent breakdown of PtdIns(4,5)P_2 and rapid formation of inositol phosphates. The latter, specifically InsP_3, may play an important role in the mechanism of cellular Ca^{2+} mobilization in the neutrophil.

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