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On the Relationship between Formylmethionyl-Leucyl-Phenylalanine Stimulation of Arachidonyl Phosphatidylinositol Turnover and Lysosomal Enzyme Secretion by Rabbit Neutrophils

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SUMMARY

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The addition of the synthetic chemotactic peptide formylmethionyl-leucyl-phenylalanine (fMet-Leu-Phe) to rabbit neutrophils promoted [^{14}C]arachidonic acid incorporation into phosphatidylinositol and lysosomal enzyme release; these two processes increased in parallel with respect to time and peptide concentration and were dependent upon extracellular Ca^{2+}. Cytochalasin B, which augments enzyme release, also enhanced fMet-Leu-Phe-evoked arachidonyl phosphatidylinositol turnover. The peptide did not alter the incorporation of labeled palmitic acid or glycerol into phosphatidylinositol, although ^{32}P turnover was increased within 2 min. Another synthetic peptide, methionyl-leucyl-phenylalanine, failed to enhance arachidonic acid incorporation into phosphatidylinositol and was unable to promote lysosomal enzyme secretion. These results support the hypothesis that fMet-Leu-Phe-induced stimulation of lysosomal enzyme release and Ca^{2+}-dependent turnover of arachidonyl phosphatidylinositol are somehow related. These findings, taken together with our previous studies demonstrating similar changes in membrane phospholipids during ACTH action on adrenocortical cells, suggest that the turnover of arachidonic acid in phospholipids mediated by a Ca^{2+}-dependent phospholipase A_2 may represent a general mechanism for producing rapid perturbations in the cell membrane during accelerated secretory activity.

INTRODUCTION

The critical role of Ca^{2+} in the sequence linking membrane activation with the extrusion of secretory product has been unequivocally established (1), but the molecular events associated with changes in membrane permeability and the fusion of the granule membrane with the plasma membrane are not yet understood.

Previous studies in our laboratory have demonstrated that ACTH expresses its steroidogenic action on cat adrenocortical cells by an activation of fatty acid turnover of membrane phospholipids (2, 3). More specifically, ACTH caused a Ca^{2+}-dependent stimulation of membrane phospholipase A_2 (EC 3.1.1.4) which brought about an increased turnover of arachidonyl phosphatidylinositol (3). This deacylation-reacylation sequence was temporally and quantitatively related to ACTH-induced steroid synthesis and release. Although the deacylation-reacylation reaction has been proposed as a critical biochemical event during membrane perturbations (4, 5), to ascribe a fundamental regulatory role to this reaction during enhanced secretory activity mandates additional inquiry into other secretory systems.

The rabbit peritoneal neutrophil has been developed as a model for studying stimulus-secretion coupling (6). Activation of lysosomal enzyme release by neutrophils can be elicited by the synthetic formylmethionyl peptides, including fMet-Leu-Phe,[1] which interact with specific receptors on the surface of the neutrophil to effect an increase in the amount of available Ca^{2+} (6–8). Moreover, in neutrophils preincubated with cytochalasin B, there is a close correlation between stimulated cation fluxes, particularly of Ca^{2+}, and secretory activity (6, 7).

The mechanism of stimulus-secretion coupling in evoked release of lysosomal enzymes is believed to be similar to that proposed for other secretory cells, wherein Ca^{2+}-induced enzyme release occurs by exocytosis (9). Accordingly, since neutrophils can serve as a useful model

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[1] The abbreviations used are: fMet-Leu-Phe, formylmethionyl-leucyl-phenylalanine; Met-Leu-Phe, methionyl-leucyl-phenylalanine.

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for investigating the secretory process, we undertook an investigation of the effects of fMet-Leu-Phe on phospholipid turnover in neutrophils. In this report, we demonstrate that the interaction of fMet-Leu-Phe with its membrane receptor produces an early Ca^{2+}-dependent activation of arachidonic acid turnover in phospholipids which bears a close resemblance to the deacylation-reacylation reaction previously demonstrated in adrenocortical cells. A preliminary account of these findings has been previously reported (10).

MATERIALS AND METHODS

Materials. The following radiolabeled materials were obtained from Amersham/Searle Corporation, Arlington Heights, Ill.: [1-^{14}C]palmitic acid (59 mCi/mmole) and [1-^{14}C]arachidonic acid (56 mCi/mmole). [^{32}P]Orthophosphoric acid (250 mCi/mmole) and [*U*-^{14}C]glycerol (43 mCi/mmole) were obtained from New England Nuclear Corporation, Boston, Mass. Other biochemicals were acquired from the following sources: cytochalasin B, fatty acid-free bovine serum albumin, *p*-nitrophenyl-*N*-acetyl-β-glucosaminide, and all phospholipid standards other than phosphatidylinositol, Sigma Chemical Company, St. Louis, Mo.; phosphatidylinositol, Supelco, Inc., Bellefonte, Pa. Earle's balanced salt solution, without Ca^{2+} and Mg^{2+}, was obtained from Flow Laboratories, Inc., Rockville, Md. Precoated thin-layer chromatographic plates (Silica gel H) were purchased from Analtech, Inc., Newark, Del. All chemicals were reagent grade.

Neutrophil preparation. Albino rabbits received i.p. injections of 400 ml of sterile 0.9% NaCl solution containing 0.1% glycogen, and the peritoneal exudate was collected in heparinized flasks 12 hr later to obtain neutrophils (for details see ref. 11). Cells used for lysosomal enzyme release assay were resuspended directly in a modified Earle's balanced salt solution supplemented to contain 0.5 mM Ca^{2+}, 0.2 mM Mg^{2+}, 20 mM 4-(2-hydroxylethyl)-1-piperazinemethanesulfonic acid (pH 7.4), and 0.025% bovine serum albumin. The cells to be used for phospholipid analysis were first washed with buffered ammonium chloride to hemolyze erythrocytes and then resuspended in the same medium as above. This medium was used throughout the remainder of the experiments, except in experiments involving Ca^{2+} deprivation, where Ca^{2+} was not added.

Radiolabel incorporation. In all incorporation studies, 4×10^6 cells were used in a final volume of 500 μl of medium incubated at 37° under an atmosphere of 95% oxygen-5% carbon dioxide. Cell suspensions were preincubated in the presence or absence of cytochalasin B (5 μg/ml) for 10 min at 37° and then were added to incubation tubes containing 250 μl of medium comprising approximately 0.2 μCi of [^{14}C]arachidonic acid, 0.8 μCi of [^{14}C]palmitic acid, 0.25 μCi of [^{14}C]glycerol, or 100 μCi of [^{32}P]orthophosphate in the presence or absence of stimulating agent. Incubations were carried out for various time intervals. The reaction was terminated by adding 3 ml of chloroform-methanol (1:2), and the lipid was extracted overnight (12). The extract was dried and resuspended in 50 μl of chloroform-methanol (9:1), and the phospholipids separated by 2-dimensional thin-layer chromatography using chloroform-methanol-acetic acid-0.1 M sodium borate (75:45:12:4.5) as the 1st-dimension system, and chloroform-acetone-methanol-acetic acid-0.1 M sodium borate (50:20:10:10:4.5) as the 2nd-dimension system (3). Unlabeled phospholipid standards were added to all extracted samples and were visualized by exposure of the developed Silica gel H plates to iodine vapor. The areas corresponding to each standard were then scraped into vials and counted for 10 min by liquid scintillation spectrometry. All values were subtracted from background counts which averaged 34 ± 1 cpm for both ^{14}C and ^{32}P. The results are expressed as average counts per minute for duplicate determinations, and generally each experiment was replicated on at least four different preparations.

Lysosomal enzyme assay. Neutrophils used in the lysosomal enzyme assay were not treated with ammonium chloride, since preliminary studies demonstrated that this treatment resulted in higher levels of basal enzyme release; however, these cells were preincubated with cytochalasin and then added to incubation tubes (4×10^6 cells) in a final incubation volume of 500 μl in the presence or absence of various concentrations of peptide. The incubation was carried out for 1–30 min and was stopped by immersing the tubes in ice; *N*-acetylglucosaminidase activity was monitored by a modification of the method of Woollen *et al.* (13). The samples were centrifuged for 5 min at 2000 × *g* at 4° and the supernatant (Supernatant I) was decanted and saved for subsequent assay. The cells were placed in Earle's medium containing 0.1% Triton X-100 for 10 min at room temperature in order to lyse the cells; the samples were then centrifuged as above to obtain Supernatant II. Assay medium (900 μl) containing 0.05 M citrate buffer (pH 4.5), fatty acid-free bovine serum albumin (0.1%), 0.1% Triton X-100, and 0.0036 M *p*-nitrophenyl-*N*-acetyl-β-glucosaminide was incubated with 200 μl of either Supernatant I or Supernatant II for 30 min at 37°. The reaction was terminated by placing the sample on ice and adding 500 μl of trichloroacetic acid (25%). The tubes were centrifuged for 5 min at 27,700 × *g* at 4°, and to 1 ml of the resulting supernatant were added 1.5 ml of ammonium hydroxide buffer (pH 10.7). Absorbance was read at 410 nm. The total amount of enzyme was determined by summation of the amount in the medium with the amount remaining in the tissue at the end of the incubation period; enzyme release is expressed as the percentage of total enzyme activity.

Statistical analysis. The statistical significance of differences between drug-treated and untreated cells was obtained by Student's *t*-test for paired samples. A value of $p < 0.05$ was taken as the criterion of statistical significance.

RESULTS

Stimulation of incorporation of [^{14}C]arachidonic acid into neutrophil phospholipids by fMet-Leu-Phe and the effect of cytochalasin. The incorporation of [^{14}C]arachidonic acid into neutrophil phospholipids is shown in Table 1. Phosphatidylcholine represented the largest amount of radiolabeled phospholipid (62%), whereas phosphatidylethanolamine (19%), phosphatidylinositol (13%), and phosphatidylserine (6%) accounted for a

TABLE 1

The effect of pretreatment with cytochalasin on the incorporation of [^{14}C]arachidonic acid into neutrophil phospholipid in the presence and absence of fMet-Leu-Phe

Rabbit neutrophils were preincubated for 10 min at 37° in the absence or presence of cytochalasin (5 μg/ml) and then transferred to tubes containing [^{14}C]arachidonic acid (0.2 μCi) with or without fMet-Leu-Phe (10^{-10} M). Incubations were carried out for 2 min and the cells were processed as described under Materials and Methods. Values for arachidonic acid incorporation in the absence or presence of cytochalasin are means ± standard error of the mean of seven and twelve different determinations, respectively, each done in duplicate. PC, Phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

Phospholipid	Arachidonic acid incorporation			
	Without cytochalasin		With cytochalasin	
	Control	fMet-Leu-Phe	Control	fMet-Leu-Phe
	cpm × 10^{-2}/incubation			
PC	9.8 ± 2.0	11.8 ± 1.2	5.9 ± 0.9	5.9 ± 0.8
PI	2.1 ± 0.5	2.9 ± 0.6	1.5 ± 0.3	3.3 ± 0.5
PS	0.9 ± 0.3	1.2 ± 0.3	0.7 ± 0.1	1.3 ± 0.2
PE	3.0 ± 0.9	3.1 ± 0.9	1.0 ± 0.2	1.3 ± 0.2

smaller percentage of label. Although cells preincubated with cytochalasin incorporated less arachidonic acid into phospholipids, a somewhat similar pattern of label distribution was observed for phosphatidylcholine (65%), phosphatidylinositol (16%), and phosphatidylserine (8%) (Table 1), suggesting that the turnover of these phospholipids was not appreciably affected by cytochalasin. On the other hand, phosphatidylethanolamine accounted for only 11% of the label, indicating that cytochalasin-treated cells incorporated less arachidonate into phosphatidylethanolamine, relative to the other phospholipids. In cells pretreated with cytochalasin, the formylmethionyl-peptide fMet-Leu-Phe produced a dose-dependent increase in label incorporation into phosphatidylinositol within 2 min, whether the values were expressed in terms of absolute counts (Fig. 1) or as a percentage of values derived from paired, untreated cells (Table 2). With peptide concentrations of 10^{-11} and 3×10^{-11} M, the increase in arachidonate incorporation was limited to phosphatidylinositol and averaged 14% and 36%, respectively (Table 2). With 10^{-10} M fMet-Leu-Phe, where a 124% increase in arachidonic acid incorporation into phosphatidylinositol was observed, a 97% increase in phosphatidylserine labeling was also demonstrable (Table 2). By contrast, cells incubated in the absence of cytochalasin showed a diminished ability to respond to 10^{-10} M fMet-Leu-Phe, with arachidonate incorporation into phosphatidylinositol increasing by only 35% as compared with 124% in the presence of cytochalasin (Table 2); no other phospholipids were significantly affected in the absence of cytochalasin (Table 2). The difference in radioactivity between stimulated and control cells treated with cytochalasin (172 ± 30 cpm) was significantly greater than the difference in radioactivity between untreated stimulated and control cells (71 ± 33 cpm) ($p < 0.05$, calculated by paired Student's *t*-test). Thus, the apparent decrease in basal arachidonate incorporation produced by cytochalasin (Table 1) cannot account for the enhancement of the stimulatory actions of fMet-Leu-Phe. To maximize responses, all subsequent experiments were carried out with cytochalasin-treated neutrophils.

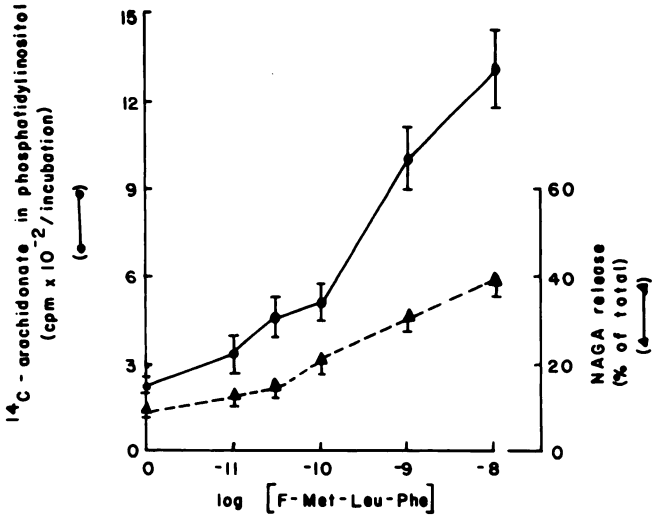

FIG. 1. *Dose-response of fMet-Leu-Phe on [^{14}C]arachidonic acid incorporation into phosphatidylinositol and lysosomal enzyme secretion*

Neutrophils pretreated with cytochalasin were incubated for 2 min with [^{14}C]arachidonic acid with or without varying concentrations of fMet-Leu-Phe and processed for phospholipid analysis as described under Materials and Methods. For *N*-acetylglucosaminidase (*NAGA*) analysis, cytochalasin-treated cells were incubated for 15 min prior to processing. All values are means ± standard error of the mean of four to eight determinations.

Comparison of phosphatidylinositol labeling and lysosomal enzyme release. The time course for arachidonic acid incorporation into phosphatidylinositol is shown in Fig. 2. From these data the rate of label incorporation was calculated by dividing the counts per minute accumulated during a given time period by the appropriate time interval. There was virtually no lag before [^{14}C]-arachidonic acid was incorporated into phosphatidylinositol, and fMet-Leu-Phe increased the rate of label incor-

TABLE 2

Dose-response of the relative change in [^{14}C]arachidonic acid content of phospholipids evoked by fMet-Leu-Phe

Cells preincubated with or without cytochalasin were added to tubes containing radiolabeled arachidonate (0.2 μCi) with or without varying concentrations of fMet-Leu-Phe. Incubations were carried out for 2 min and the cells were processed as described under Materials and Methods. Values for fMet-Leu-Phe-treated cells are represented as a percentage of values derived from paired untreated cells and are the means ± standard error of the mean of at least five different determinations, each done in duplicate. PC, Phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

Phospholipid	fMet-Leu-Phe			
	10^{-11} M	3×10^{-11} M	10^{-10} M	10^{-10} M without cytochalasin
PC	105 ± 5	104 ± 3	102 ± 3	117 ± 9
PI	114 ± 3[a]	136 ± 9[a]	224 ± 24[a]	135 ± 11[a]
PS	102 ± 8	93 ± 10	197 ± 25[a]	123 ± 27
PE	92 ± 12	100 ± 8	131 ± 10	111 ± 11

[a] $p < 0.05$ as determined by paired Student's t-test.

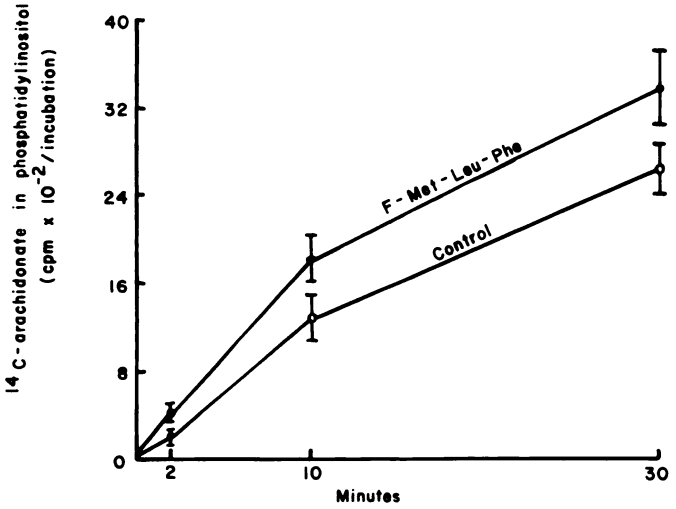

FIG. 2. *Effect of fMet-Leu-Phe on the time course of [^{14}C]arachidonic acid incorporation into phosphatidylinositol*

Cells pretreated with cytochalasin were incubated with radiolabel in the presence or absence of fMet-Leu-Phe (10^{-10} M), and label incorporation into phosphatidylinositol was measured at various times. All values are means ± standard error of the mean of four to eleven different experiments, each performed in duplicate.

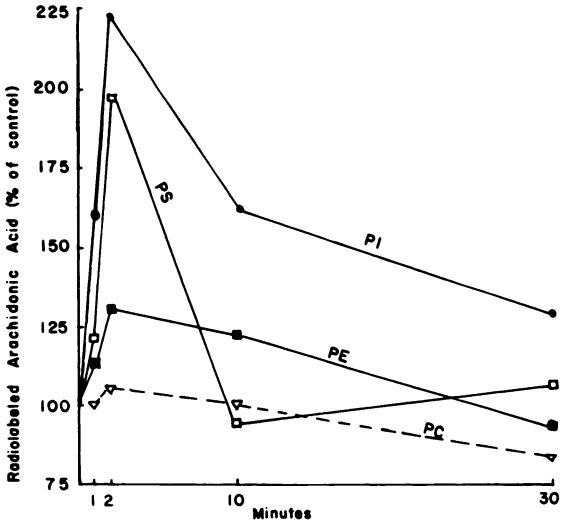

FIG. 3. *Relative changes in [^{14}C]arachidonic acid incorporation into neutrophil phospholipids elicited by fMet-Leu-Phe*

Cells pretreated with cytochalasin were incubated for various times with radiolabel in the presence or absence of fMet-Leu-Phe (10^{-10} M). Incorporation of radiolabel into phospholipids is expressed as a percentage of that in unstimulated cells. All values are means of at least four different determinations, but for the sake of clarity the bars for the standard error of the mean, which was always less than 15%, have been omitted. Paired *t*-test analysis gave a significant difference ($p < 0.05$) in label incorporation into phosphatidylinositol between peptide-treated and untreated cells at each time point; label incorporation into phosphatidylserine was enhanced at 2 min ($p < 0.05$). *PC,* Phosphatidylcholine; *PS,* phosphatidylserine; *PE,* phosphatidylethanolamine; *PI,* phosphatidylinositol.

poration within the first 2 min from 75 to 199 cpm/min (Fig. 2). The stimulated rate of label incorporation gradually declined, such that during the 10th–30th min of incubation the average rate in fMet-Leu-Phe-treated cells fell to 76 cpm/min, as compared with 68 cpm/min in unstimulated cells. When values from stimulated cells were represented as a percentage of values derived from control cells at each point, a significant increase in label incorporation into phosphatidylinositol was observed within 1 min, reaching peak levels at 2 min (Fig. 3). Peak stimulation of phosphatidylserine labeling was also demonstrable at 2 min (Fig. 3). The 31% increase in the labeling of phosphatidylethanolamine at 2 min was not statistically significant, and phosphatidylcholine exhibited no alterations in the labeling of arachidonic acid during exposure to fMet-Leu-Phe over the 30-min time period (Fig. 3). The time course of lysosomal enzyme release elicited by fMet-Leu-Phe (10^{-10} M) is depicted in Fig. 4. A significant increase in *N*-acetylglucosaminidase release was demonstrable within 1 min which reached completion within 2 min. By contrast, no increase in soluble lactic dehydrogenase release was observed even after 15 min (data not shown). A comparison of the dose-response relationship for fMet-Leu-Phe stimulation of phosphatidylinositol labeling and enzyme release is illustrated in Fig. 1. Values for dependence on peptide concentration of stimulated arachidonic acid incorporation and enzyme release were very similar. Thus, at the lowest peptide concentration (10^{-11} M) at which significant increases in label incorporation were detectable, significant increases in enzyme release were also initially observed. Both parameters continued to rise with increasing peptide concentrations as high as 10^{-8} M (Fig. 1).

Another synthetic peptide, Met-Leu-Phe, failed to affect arachidonic acid incorporation into phosphatidylinositol or any of the other phospholipids examined. Thus, values for relative incorporation of [^{14}C]arachidonic acid into neutrophil phosphatidylinositol in the presence of Met-Leu-Phe (10^{-10} M) after 1, 10, and 30 min were 103 ± 9%, 106 ± 7%, and 91 ± 8% of control cells, respectively ($n = 5$). Met-Leu-Phe (10^{-10}–10^{-9} M) also did not stimulate lysosomal enzyme release, as evidenced by the fact that after a 15-min exposure to the peptide, enzyme

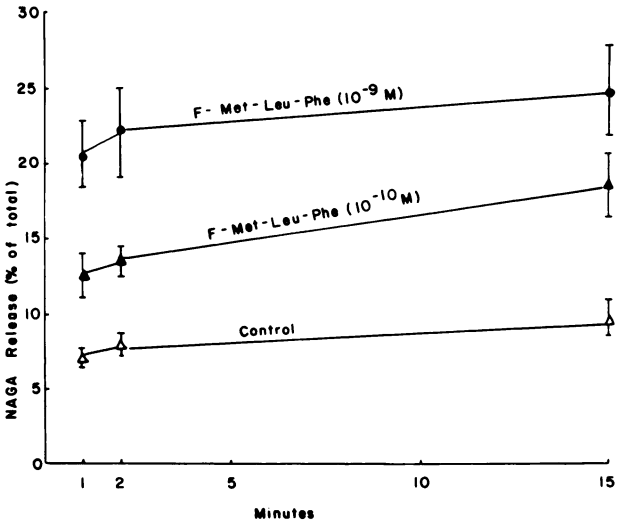

FIG. 4. *Time course of N-acetylglucosaminidase (NAGA) release*

Neutrophils pretreated with cytochalasin were incubated with or without fMet-Leu-Phe for various times. Values are means ± standard error of the mean of four different determinations.

TABLE 3

Effect of Ca^{2+} deprivation on fMet-Leu-Phe-induced arachidonate labeling of phosphatidylinositol and lysosomal enzyme release

After a 10-min incubation period with cytochalasin in either Ca^{2+}-free or Ca^{2+}-containing medium, cells were incubated for 2 min with 0.2 μCi [^{14}C]arachidonate with or without fMet-Leu-Phe (10^{-10} M) in the presence or absence of Ca^{2+}. Incubations were carried out for 2 min and the cells were processed for phospholipid analysis as described under Materials and Methods. For lysosomal enzyme assay, incubations were carried out for 15 min and the samples were processed as described under Methods. Values for arachidonic acid incorporation are means ± standard error of the mean of four different determinations, each done in duplicate. Values for lysosomal enzyme release are means ± standard error of the mean of three and ten determinations in Ca^{2+}-free and Ca^{2+}-containing media, respectively. Values for fMet-Leu-Phe-treated cells are also represented as a percentage of values derived from paired untreated cells (percentage of control). PI, phosphatidylinositol.

Addition	Arachidonic acid incorporation into PI			*N*-Acetylglucosaminidase release		
	Control	fMet-Leu-Phe	% of control	% of total		% of Control
				Control	fMet-Leu-Phe	
	cpm × 10^{-2}/incubation					
None	1.8 ± 0.2	2.0 ± 0.1	118 ± 6	7.3 ± 0.9	7.3 ± 0.7	101 ± 7
0.5 mM Ca^{2+}	3.0 ± 0.7	5.4 ± 1.3	180 ± 6	10.6 ± 0.8	21.9 ± 3.4	185 ± 19

secretion was 10.8 ± 1.4% (n = 4), which was not significantly different from the mean control value of 10.3%.

Table 3 shows that, when cytochalasin-treated neutrophils were washed and incubated in Ca^{2+}-free medium, the ability of fMet-Leu-Phe (10^{-10} M) to enhance arachidonyl phosphatidylinositol turnover was profoundly depressed. Similarly, Ca^{2+} deprivation completely blocked lysosomal enzyme release evoked by fMet-Leu-Phe (Table 3).

Incorporation of [^{14}C]palmitic acid, ^{32}P, and [^{14}C]-glycerol into neutrophil phospholipids. The saturated fatty acid palmitic acid was also incorporated into neutrophil phospholipids. The pattern of palmitate distribution was similar to that of arachidonate distribution, with phosphatidylcholine accounting for a large proportion of the label (75%) (Table 4); however, the phosphatidylinositol fraction incorporated much less palmitate (3%) than arachidonate (16%) (Table 4). Table 4 also shows that fMet-Leu-Phe (10^{-10} M) did not significantly enhance the incorporation of palmitic acid into phosphatidylinositol, although the peptide did elicit a 38% increase in palmitate incorporation into phosphatidylethanolamine (Table 4).

The relative distribution of ^{32}P in neutrophil phospholipids was markedly different from the distribution of [^{14}C]arachidonic acid. Thus, 80% of the label was associated with the phosphatidylethanolamine fraction, with phosphatidylcholine, phosphatidylinositol, and phosphatidylserine accounting for 8%, 8%, and 3% of the label, respectively (Table 5). fMet-Leu-Phe (10^{-10} M) increased incorporation of ^{32}P into phosphatidylinositol by 71% after 2 min (Table 5); label incorporation subsequently decreased to 22 ± 3% above control levels after 10 min. In the presence of the peptide, the incorporation of ^{32}P into phosphatidylcholine and phosphatidylserine was slightly greater than control values, although not statistically significant (Table 5).

To determine whether *de novo* phospholipid synthesis rather than turnover might explain the stimulation of arachidonate incorporation into phosphatidylinositol, neutrophils were exposed to [^{14}C]glycerol for 2 min. Although phosphatidylinositol accounted for 94% of the label, fMet-Leu-Phe failed to elicit any additional conversion of glycerol to phosphatidylinositol. Thus, label incorporation values in the absence and presence of fMet-Leu-Phe (10^{-10} M) were 1661 ± 105 and 1546 ± 138 cpm, respectively (n = 3).

TABLE 4

Effect of fMet-Leu-Pheon incorporation of [^{14}C]palmitic acid into neutrophil phospholipids

Cytochalasin-treated cells were added to tubes containing 0.8 μCi of [^{14}C]palmitic acid in the presence or absence of fMet-Leu-Phe (10^{-10} M). Incubations were carried out for 2 min and the cells were processed as described under Materials and Methods. Values for palmitic acid incorporation are means (± standard error of the mean) of five different determinations, each done in duplicate. Values for fMet-Leu-Phe-treated cells are also represented as a percentage of values derived from paired untreated cells. PC, Phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

Phospholipid	Palmitic acid incorporation		
	Control	fMet-Leu-Phe	% of control
	cpm × 10^{-2}/incubation		
PC	15.9 ± 4.3	17.9 ± 4.5	115 ± 7
PI	0.7 ± 0.2	0.9 ± 0.2	113 ± 4
PS	1.4 ± 0.2	1.2 ± 0.2	93 ± 7
PE	3.2 ± 0.6	4.4 ± 0.9	138 ± 9[a]

[a] $p < 0.05$ as determined by paired Student's t-test.

TABLE 5

Effect of fMet-Leu-Phe on incorporation of [^{32}P]orthophosphate into neutrophil phospholipids

Cytochalasin-treated cells were added to tubes containing 100 μCi of [^{32}P]orthophosphate in the presence or absence of fMet-Leu-Phe (10^{-10} M). Incubations were carried out for 2 min and the cells were processed as described under Materials and Methods. Values shown are the means (± standard error of the mean) of five different determinations, each done in duplicate. Values for fMet-Leu-Phe-treated cells are also represented as a percentage of values derived from paired untreated cells. PC, Phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

Phospholipid	[^{32}P]Orthophosphate incorporation		
	Control	fMet-Leu-Phe	% of control
	cpm × 10^{-2}/incubation		
PC	2.8 ± 0.8	2.6 ± 0.8	127 ± 17
PI	2.8 ± 0.8	3.6 ± 1.0	171 ± 19[a]
PS	1.2 ± 0.3	1.7 ± 0.5	116 ± 19
PE	27.6 ± 5.7	25.4 ± 8.3	103 ± 4

[a] $p < 0.05$ as determined by paired Student's t-test.

DISCUSSION

The present study has demonstrated an association between the ability of fMet-Leu-Phe to stimulate the incorporation of arachidonic acid into phosphatidylinositol and to promote lysosomal enzyme release. This conclusion is based upon the following observations: (a) fMet-Leu-Phe-evoked arachidonic acid incorporation and lysosomal enzyme release increased *pari passu* with respect to time and peptide concentration; (b) cytochalasin, which is known to enhance lysosomal enzyme release elicited by fMet-Leu-Phe (7), increased arachidonyl phosphatidylinositol turnover; (c) Met-Leu-Phe, which was unable to stimulate enzyme release in the concentration employed, also failed to enhance the incorporation of arachidonic acid into phosphatidylinositol; (d) Ca^{2+} deprivation blocked both lysosomal enzyme release and arachidonyl phosphatidylinositol turnover. The importance of Ca^{2+} in this biochemical reaction is reinforced by the ability of Ca^{2+} to mimic the effects of fMet-Leu-Phe on phospholipid turnover using the divalent cation ionophore A23187 (10) and by the knowledge that cytochalasin, which promoted arachidonic acid turnover, also greatly enhances the rate and extent of Ca^{2+} uptake induced by fMet-Leu-Phe, as well as lysosomal enzyme release (7).

The proposition that a deacylation-reacylation mechanism mediated by a Ca^{2+}-dependent phospholipase A_2 is responsible for fMet-Leu-Phe-induced stimulation of arachidonate turnover is considered the most likely interpretation of our results on the basis of several lines of evidence. Arachidonic acid is primarily incorporated into position 2 of phospholipids, and turnover of this unsaturated fatty acid has generally been interpreted as a monitor of phospholipase A_2 activity (14). Both components of the deacylation-reacylation cycle, a membrane-bound phospholipase A_2 and an acyl-CoA transferase, have been identified in rabbit neutrophils by Elsbach (15) and Franson *et al.* (16), and the release of arachidonic acid and the synthesis of prostaglandins by fMet-Leu-Phe and A23187 have been demonstrated in neutrophils of various species, including rabbit (17–20). fMet-Leu-Phe did not alter the incorporation into phosphatidylinositol of glycerol or the saturated fatty acid palmitic acid, which is incorporated preferentially into position 1 of phospholipids (4). These findings support the notion that phosphatidylinositol turnover was not a reflection of *de novo* phosphatidylinositol synthesis during this early stage of peptide action.

Thus, the most reasonable interpretation of our data encompasses the concept that fMet-Leu-Phe activates a membrane-bound phospholipase A_2 in neutrophils; the resulting deacylation of phosphatidylinositol would precede the acylation of lysophosphatidylinositol by arachidonic acid via an acyltransferase-mediated reaction. A similar preference for phosphatidylinositol as a substrate was found in our previous studies during phospholipase A_2 activation by adrenocorticotropin on isolated adrenocortical cells (3) as well as during phospholipase A_2 activation by thyrotropin in the thyroid gland (21). The key role for phosphatidylinositol may be ascribed to the preference of neutrophil phospholipase and/or acyltransferase for this particular phospholipid. The reports demonstrating that membrane fractions from adrenal glands, brain, and liver preferentially transfer arachidonyl CoA to lysophosphatidylinositol (22–24) suggest that the reacylation component is responsible, at least in part, for the relative specificity for phosphatidylinositol. It should also be mentioned that, although the proposed deacylation-reacylation sequence exhibited a preference for phosphatidylinositol at threshold concentrations of fMet-Leu-Phe in the presence of cytochalasin, and at higher peptide concentrations (10^{-10} M) in the absence of cytochalasin, fMet-Leu-Phe (10^{-10} M) stimulated arachidonate incorporation into phosphatidylserine derived from neutrophils pretreated with cytochalasin. These data indicate that, with a greater degree of membrane activation brought about by cytochalasin pretreatment plus high peptide concentrations, other phospholipids become substrates for this reaction.

fMet-Leu-Phe also transiently enhanced the turnover of ^{32}P into phosphatidylinositol within 2 min. In contrast to other systems, this fMet-Leu-Phe-induced ^{32}P turnover is dependent upon extracellular Ca^{2+} (25) and can be stimulated by A23187.[2] A Ca^{2+}-activated phospholipase C may account for this effect, but direct evidence to support this supposition is still lacking. However, a Ca^{2+}-dependent activation of phospholipase C has been demonstrated during the release reaction of the blood platelet (26, 27).

In summary, the present study has shown that the activation of cell surface receptors of neutrophils by fMet-Leu-Phe is accompanied by an increase in available Ca^{2+} to stimulate phospholipase A_2, thereby initiating the deacylation-reacylation cycle. It remains to be determined whether the changes in phospholipids which have been demonstrated in this investigation are associated with alterations in membrane phospholipids prior to secretion, with events associated with the release process itself, or with some other reaction unrelated to secretion. Nevertheless, a most interesting aspect of this study relates to the parallels with our previous studies on adrenocorticotropin-induced arachidonic acid turnover in cat adrenocortical cells, where an alteration in the turnover of arachidonyl phosphatidylinositol via a hormone-sensitive Ca^{2+}-dependent phospholipase A_2 was found to be an early and specific event in the action of adrenocorticotropin (3, 28). These results led us to conclude that adrenocorticotropin expresses its effects by a Ca^{2+}-dependent activation of fatty acid turnover of membrane phospholipids which leads to functional alterations in the membrane. The present report suggests that in the rabbit neutrophil a similar mechanism is operative.

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