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Calcium influx stimulates a second pathway for sustained diacylglycerol production in leukocytes activated by chemoattractants

(stimulus–response coupling/receptors/G proteins/inositolphospholipid metabolism)

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ABSTRACT **Metabolic pathways involved in the activation of polymorphonuclear leukocytes (PMNs) were characterized by using chemoattractants with equivalent chemotactic activity but widely disparate ability to stimulate superoxide production [*N*-formylmethionylleucylphenylalanine (fMet-Leu-Phe) >> leukotriene B_4]. Leukotriene B_4 stimulated a low level of superoxide production that plateaued at 60 sec, whereas with fMet-Leu-Phe the response continued to increase for 5 min. Both agents produced equivalent initial rises in diacylglycerol ($acyl_2Gro$) (≤30 sec); however, only fMet-Leu-Phe induced a second increase of $acyl_2Gro$ peaking at *ca.* 120 sec. Both chemoattractants also caused an equivalent initial (≤10 sec) rise in intracellular calcium; however, the elevation induced by fMet-Leu-Phe was more sustained. We sought to determine the biochemical mechanisms underlying these discrepancies. Superoxide production and the second phase of $acyl_2Gro$ generation were both inhibited *ca.* 56% by depleting extracellular calcium or *ca.* 79% by buffering intracellular calcium. Cytochalasin B greatly enhanced the respiratory burst, $acyl_2Gro$ production, and calcium influx, but not inositolphospholipid turnover in PMNs stimulated with chemoattractants. These data indicate that sequential metabolic pathways activate the respiratory burst in PMNs stimulated by chemoattractants. The response is initiated by inositolpolyphospholipid hydrolysis, which results in rapid (≤5 sec) calcium mobilization from intracellular stores and $acyl_2Gro$ release (peak at *ca.* 30 sec). To fully activate the respiratory burst, the chemoattractant must also trigger calcium influx, which leads to a sustained cytosolic calcium elevation. This supports a prolonged new phase of $acyl_2Gro$ production that is independent of inositolphospholipid hydrolysis and is correlated with superoxide production.**

Activation of polymorphonuclear leukocytes (PMNs) by chemoattractants is dependent upon a G protein coupled to a phospholipase C, which hydrolyzes phosphatidylinositol 4,5-bis(phosphate) ($PtdIns P_2$) to form inositol 1,4,5-tris(phosphate) ($Ins P_3$) and 1,2-diacylglycerol ($acyl_2Gro$) (for reviews, see refs. 1–3). Release of 1,4,5-$Ins P_3$ mobilizes intracellular calcium (4) and $acyl_2Gro$ activates protein kinase C (5). Protein kinase C can mediate superoxide generation by leukocytes, as suggested by experiments in which active phorbol esters (6) or cell-permeant $acyl_2Gro$ (7) induced a respiratory burst. Although chemoattractants as a class utilize the receptor–G protein–inositolphospholipid pathway to activate leukocytes, they have widely disparate abilities to stimulate the respiratory burst (8). For example, fMet-Leu-Phe and leukotriene B_4 (LTB_4) are both potent chemoattractants (EC_{50}, *ca.* 10^{-9} M), but the potency for generation of superoxide is markedly less for LTB_4 (EC_{50}, ≥1 μM) than for fMet-Leu-Phe (EC_{50}, *ca.* 0.02 μM), and the maximal LTB_4-induced response is approximately one-third that of fMet-Leu-Phe (8–11). To define the biochemical pathway involved in activating the respiratory burst, we compared the effects of these two stimuli on $acyl_2Gro$ production, calcium mobilization, and inositolphospholipid metabolism under conditions where the superoxide response was either potentiated with cytochalasin B or diminished by extra- or intracellular calcium chelators (12–14).

The results of these studies suggest that chemoattractants activate the respiratory burst via sequential pathways. The initial pathway involves inositolphospholipid hydrolysis, which through its second messengers, if sufficiently sustained, can stimulate calcium influx. Sustained calcium mobilization from extracellular sources can then trigger a second phase of $acyl_2Gro$ production from a source other than hydrolysis of inositol-containing lipids. Only the first source of $acyl_2Gro$ production is activated effectively in LTB_4-stimulated cells, accounting for the poor ability of this chemoattractant to stimulate a respiratory burst.

EXPERIMENTAL PROCEDURES

Materials. Unless stated otherwise, all drugs and reagents were obtained from previously described sources (6–9). Dioleoylphosphatidylglycerol was obtained from Avanti Polar Lipids and silica gel 60 F_{254} thin-layer chromatography plates were from EM Science (Cincinnati, OH). *sn*-1,2-Dioleoylglycerol (diolein) and *Escherichia coli* strain N4830/pJW10 membranes (15) were the gifts of R. M. Bell and W. R. Bishop (Duke University, Durham, NC).

Cell Preparation. PMNs from healthy volunteers were isolated (≥95% purity) as described (7) and resuspended at 2 × 10^7 cells per ml in Hanks' balanced salt solution containing 10 mM Hepes and 4.2 mM $NaHCO_3$ (pH 7.4) (HHBSS). To deplete extracellular calcium, cells were resuspended in HHBSS without calcium and magnesium ($HHBSS^{2-}$) to which 1 mM EGTA and 1 mM magnesium were added. Intracellular calcium was chelated by incubating cells in HHBSS in the presence of 50 μM Quin-2 AM at 37°C for 40 min (16). The cells were then washed and resuspended in $HHBSS^{2-}$ including 1 mM calcium and 1 mM magnesium. Cells were kept on ice until time of assay.

Quantitation of $acyl_2Gro$. Aliquots of cells (0.5 ml) were added to 0.25 ml of assay buffer (HHBSS or $HHBSS^{2-}$ in calcium-depletion experiments) containing 25 μl of cytocha-

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Abbreviations: $acyl_2Gro$, 1,2-diacyl-*sn*-glycerol(s); fMet-Leu-Phe, *N*-formylMet-Leu-Phe; LTB_4, leukotriene B_4; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear leukocyte; $Ins P_3$, inositol tris(phosphate); $PtdIns P_2$, phosphotidylinositol 4,5-bis(phosphate); $Ins P_4$, inositol 1,3,4,5-tetrakis(phosphate); Ins*P*, inositol mono(phosphate); $Ins P_2$, inositol bis(phosphate).

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lasin B (10 μM final) or an equivalent dilution of its diluent Me_2SO. The cells were prewarmed at 37°C for 5 min before addition of 25 μl of chemoattractant or diluent control. Reactions were terminated with 3 ml of chloroform/methanol (1:2, vol/vol). Lipids were extracted by a modified method of Bligh and Dyer (17) and $acyl_2Gro$ levels in the crude lipid extracts were quantitated as described by Preiss *et al.* (41), except that 25 mM dioleoylphosphatidylglycerol was substituted for 5 mM cardiolipin.

Other Assay Procedures. Superoxide generation was measured by the reduction of cytochrome *c*, and intracellular free calcium levels were monitored with Fura-2 AM (7, 13, 19–21). Inositolphospholipids were labeled by culturing cells overnight in medium containing [^{3}H]*myo*-inositol at 20 μCi/ml (1 Ci = 37 GBq) (22). PMNs were then washed, resuspended (6×10^7 cells per ml) in HHBSS with 20 mM $LiCl_2$, and prewarmed for 5 min at 37°C with 10 μM cytochalasin B or an equivalent dilution of Me_2SO before addition of 10× stimuli (8). Reactions were terminated, and inositol phosphates were analyzed by HPLC (8, 23).

Statistical Analysis. Data are expressed as the mean ± SEM. Significance ($P < 0.05$) was determined by Student's paired *t* test.

RESULTS

$acyl_2Gro$ Production, Calcium Mobilization, and Superoxide Generation in PMNs Stimulated with fMet-Leu-Phe or LTB_4. The $acyl_2Gro$ mass levels in cells stimulated with fMet-Leu-Phe or LTB_4 increased in a dose-dependent manner (EC_{50} for fMet-Leu-Phe of *ca.* 5 nM and EC_{50} for LTB_4 of 50 nM). $acyl_2Gro$ concentrations reached equivalent levels by 30 sec in cells stimulated with 1 μM fMet-Leu-Phe or LTB_4. However, after 30 sec, $acyl_2Gro$ levels plateaued and gradually fell in LTB_4-stimulated cells, whereas $acyl_2Gro$ production continued to increase in cells stimulated with fMet-Leu-Phe. Maximal $acyl_2Gro$ levels occurred at 2 min, and $acyl_2Gro$ levels in fMet-Leu-Phe-stimulated cells remained significantly elevated above those in LTB_4-stimulated cells for at least 10 min (Fig. 1*A*). Pretreatment with 10 μM cytochalasin B significantly enhanced $acyl_2Gro$ production in response to either chemoattractant after 10 sec in comparison to PMNs stimulated with either chemoattractant alone (Fig. 1*A* vs. 1*B*). In the cytochalasin B-pretreated cells, the initial (20–30 sec) amount of $acyl_2Gro$ production induced by LTB_4 was equivalent to that of fMet-Leu-Phe, but the $acyl_2Gro$ production did not continue to rise with time in the LTB_4-stimulated cells (Fig. 1*B*). Baseline $acyl_2Gro$ levels did not vary significantly for the entire 10 min in unstimulated PMNs with or without cytochalasin B (data not shown).

Concurrent measurements of superoxide production and intracellular calcium concentrations were made on cells from the same donors. Results for superoxide production paralleled the $acyl_2Gro$ results described above. Without cytochalasin B, LTB_4-induced superoxide production peaked at *ca.* 50% of fMet-Leu-Phe levels (Fig. 1*C*). With cytochalasin B, fMet-Leu-Phe-induced superoxide production was enhanced and was 3-fold greater than LTB_4-stimulated cells containing cytochalasin B (Fig. 1*D*). As with the $acyl_2Gro$ responses, the early production of superoxide was equivalent in cells stimulated with the same dose of either chemoattractant, whereas the continued rise in superoxide production was more pronounced in fMet-Leu-Phe-stimulated cells.

Both chemoattractants promoted a rapid rise in intracellular calcium, which peaked by *ca.* 10 sec (Fig. 2). However, as noted previously (8, 9, 23), the cytosolic calcium rise returned to baseline at a slower rate in fMet-Leu-Phe vs. LTB_4-stimulated cells. The addition of cytochalasin B resulted in a pronounced second elevation in intracellular calcium that peaked at 5 min in fMet-Leu-Phe-stimulated cells. As was the case for superoxide and $acyl_2Gro$ production, cytochalasin B prolonged the calcium elevation above baseline in LTB_4-stimulated cells but did not stimulate the secondary rise in intracellular calcium.

Requirement for Extracellular and Intracellular Calcium for $acyl_2Gro$ Production and Superoxide Generation. To investigate the apparent relationship between intracellular calcium levels and $acyl_2Gro$ production, extracellular calcium was chelated with EGTA. In the presence of cytocha-

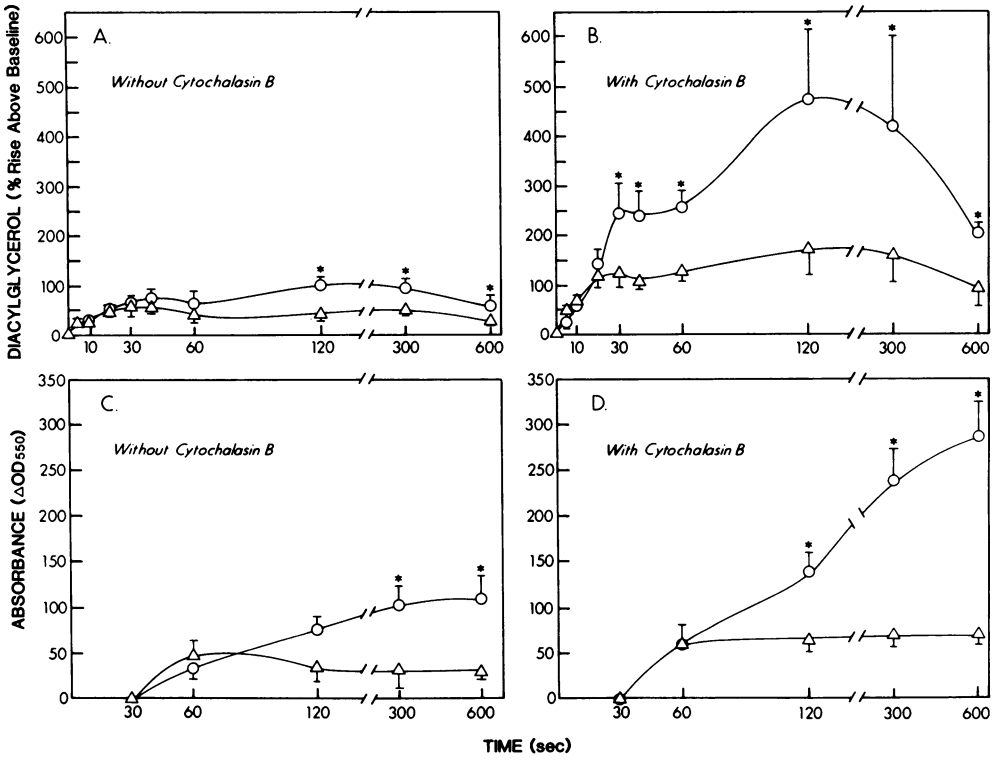

FIG. 1. The effect of fMet-Leu-Phe and LTB_4 on $acyl_2Gro$ and superoxide production. In concurrent experiments, $acyl_2Gro$ and superoxide anion production were measured in PMNs stimulated with 1 μM fMet-Leu-Phe (○) vs. 1 μM LTB_4 (△) in the absence (*A* and *C*) or presence (*B* and *D*) of 10 μM cytochalasin B. $acyl_2Gro$ production (*A* and *B*) is expressed as the percent rise above baseline (180.7 ± 15.4 pmol per 10^7 cells) ($n = 4$). Superoxide generation is expressed as the mean net absorbance of cytochrome $c \times 10^{-3}$ ± SEM. Total superoxide formed in response to fMet-Leu-Phe and LTB_4 was 3.7 ± 8.8 and 1.5 ± 6.5 nmol per 10^7 cells, respectively, in the absence of cytochalasin B, and 9.8 ± 1.3 and 2.4 ± 4.1 nmol per 10^7 cells in the presence of cytochalasin B. Significant differences between fMet-Leu-Phe and LTB_4 ($P < 0.05$) are indicated by *.

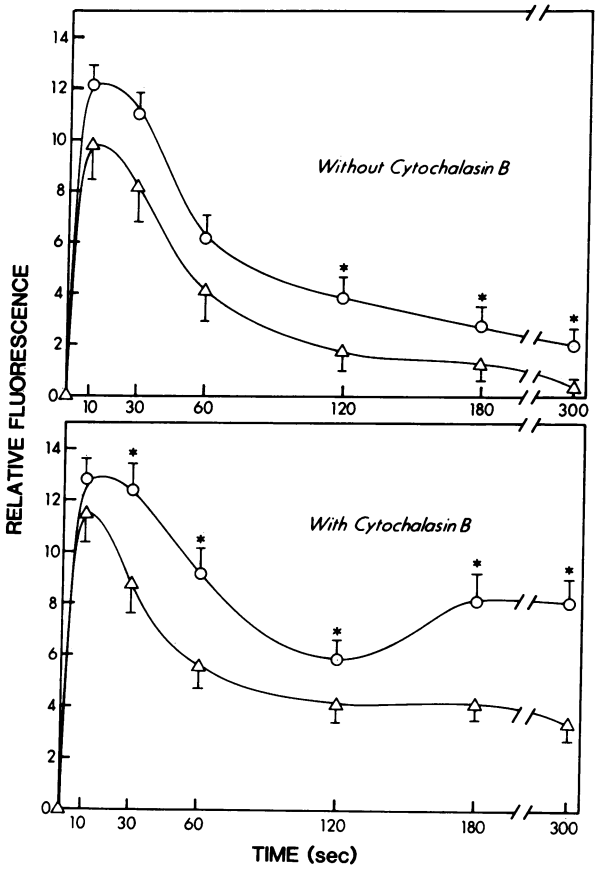

FIG. 2. The effect of fMet-Leu-Phe, LTB_4, and cytochalasin B on intracellular calcium concentrations. Fura-2-loaded PMNs were stimulated with 1 μM fMet-Leu-Phe (○) or 1 μM LTB_4 (△) in the absence (*Upper*) or presence (*Lower*) of 10 μM cytochalasin B. Results shown represent the mean relative fluorescence ± SEM for eight separate experiments from different donors. Significant differences between fMet-Leu-Phe and LTB_4 ($P \geq 0.05$) are indicated by *.

lasin B, fMet-Leu-Phe induced equivalent $acyl_2Gro$ elevations at 5 and 10 sec in the presence or absence of extracellular calcium (Fig. 3 *Left*). However, $acyl_2Gro$ production then lagged in calcium-free media and reached a peak of only

Table 1. Effect of buffering $[Ca]_i$ on $acyl_2Gro$ production

Time, sec	Control, % change*	Quin-2, % change*
5	33.1 ± 22.9	52.2 ± 21.2
10	69.2 ± 14.5	67.3 ± 38.6
120	562.4 ± 96.8	101.3 ± 32.2†

Cells were loaded with Quin-2 AM to buffer cytoplasmic calcium changes. Cells were pretreated for 5 min with 10 μM cytochalasin B and then stimulated with 1 μM fMet-Leu-Phe. $acyl_2Gro$ mass was quantitated as described.
*Results are expressed as the mean percent rise above baseline $acyl_2Gro$ levels (165.8 ± 10.3 pM per 10^7 cells) ± SEM.
†Indicates $P \leq 0.005$ (compared to control value at 120 sec).

43.8 ± 6.5% (n = 8) of that obtained in calcium-containing media. The second phase of calcium mobilization seen in stimulated PMNs treated with cytochalasin B (see Fig. 2*B*) was similarly absent in the presence of EGTA (data not shown). In concurrent measurements, it was shown that depletion of extracellular calcium also reduced fMet-Leu-Phe-induced superoxide generation to only 42 ± 10% (n = 6) of the response seen in calcium-containing medium (Fig. 3 *Right*).

PMNs were loaded with sufficiently high concentrations (50 μM) of the calcium-chelator Quin-2 to prevent elevation of cytosolic calcium (13, 16). This pretreatment had no effect on fMet-Leu-Phe-stimulated $acyl_2Gro$ production at 5 or 10 sec; however, the peak $acyl_2Gro$ response (2 min) was markedly inhibited [78.4 ± 9.4% (n = 5)] (Table 1). Similarly, fMet-Leu-Phe-induced superoxide production in cells loaded with 50 μM Quin-2 was inhibited by 72 ± 17% (n = 5, $P < 0.05$) at 10 min. Phorbol 12-myristate 13-acetate (PMA) (100 ng/ml)-induced superoxide production at 10 min was not affected by either EGTA or Quin-2, showing that these treatments did not disrupt the superoxide-generating system.

Effect of Cytochalasin B on Inositol Phosphate Production. To determine whether the increased elevations of calcium and $acyl_2Gro$ in cytochalasin B-treated cells resulted from enhanced release of calcium-mobilizing inositol phosphates, inositol phosphate metabolism was measured in PMNs labeled overnight with [^{3}H]*myo*-inositol (Fig. 4). In fMet-Leu-Phe-stimulated cells, cytochalasin B pretreatment did not alter the kinetics or magnitude of production of 1,4,5-InsP_3 or of its metabolites inositol tetrakis(phosphate) (InsP_4) or

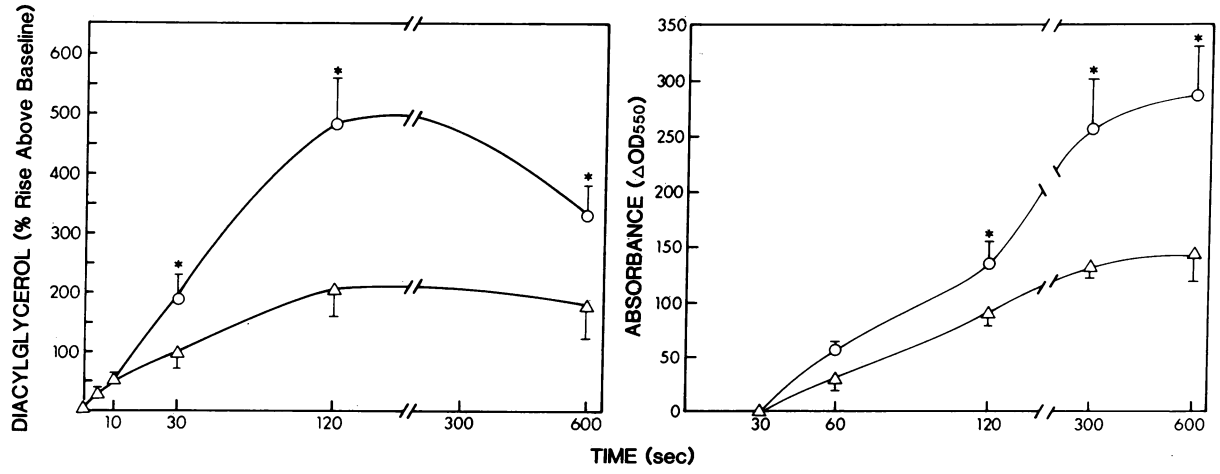

FIG. 3. Effect of extracellular calcium depletion on $acyl_2Gro$ production and superoxide anion generation. PMNs suspended in HHBSS (○) or HHBSS^{2-} plus 1 mM EGTA and magnesium (△) (see *Methods*) were stimulated with 1 μM fMet-Leu-Phe after 5-min pretreatment with 10 μM cytochalasin B at 37°C. $acyl_2Gro$ and superoxide production were measured as described. $acyl_2Gro$ production is expressed as the mean percent rise above baseline (165.8 ± 10.1 pmol per 10^7 cells) ± SEM (n = 8). Superoxide is expressed as the mean net absorbance of cytochrome $c \times 10^{-3}$ ± SEM. Total superoxide produced was 9.8 ± 1.5 and 4.9 ± 0.5 nmol per 10^7 cells in the presence or absence of extracellular calcium, respectively. Significant differences ($P \leq 0.05$) in the presence or absence of extracellular calcium are indicated by *.

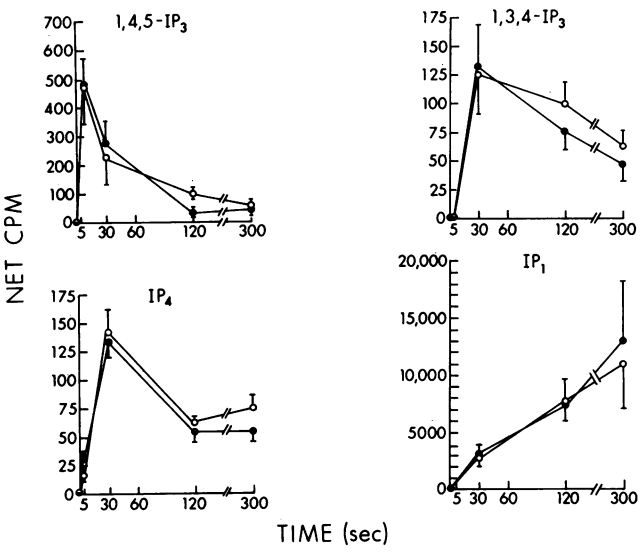

FIG. 4. Effect of cytochalasin B on inositol phosphate production in PMNs stimulated with 1.0 μM fMet-Leu-Phe. Trichloroacetic acid-soluble material from [^{3}H]*myo*-inositol-labeled PMNs was analyzed as described (23). Net cpm (stimulated minus buffer control) are shown for cells pretreated with 10 μM cytochalasin B (●) or an equivalent concentration of Me_2SO (○). Results represent the mean cpm ± SEM for four separate experiments with different donors. IP, inositol phosphate.

1,3,4-InsP_3 (Fig. 4). Similarly cytochalasin B did not affect total inositol mono(phosphate) (Ins$P$) (Fig. 4) or the relative proportion of the 4-Ins$P$ vs. the 1- and 3-Ins$P$ isomers (24) (data not shown), suggesting that neither InsP_3 degradation nor phosphatidylinositol hydrolysis was affected by cytochalasin B. Experiments run in parallel on PMNs incubated overnight confirmed that cytochalasin B enhanced acyl$_2$Gro levels as seen in freshly isolated cells stimulated with fMet-Leu-Phe (data not shown).

DISCUSSION

The initial steps involved in leukocyte activation by chemoattractant receptors require the participation of a G protein to activate PtdInsP_2 hydrolysis via a membrane-associated phospholipase C (1–3). The metabolic processes involved beyond these events are complex and are not completely understood. The chemoattractant fMet-Leu-Phe generates substantially more superoxide in PMNs than does LTB_4 (8–11), although both agents are equally effective chemoattractants. This discrepancy provides a useful means to define the relevant pathways involved in activation of the respiratory burst. By measuring acyl$_2$Gro levels, calcium mobilization, inositolphospholipid metabolism, and superoxide generation, we have been able to further characterize the mechanism of PMN activation by chemoattractants.

In the present study, time course measurements revealed that the initial (≤30 sec) net acyl$_2$Gro levels were equivalent after stimulation with either chemoattractant at a time when superoxide release was not yet detectable. The later rise of acyl$_2$Gro production (≥30 sec) was significantly lower in response to LTB_4 than to fMet-Leu-Phe and thus correlated with the poor superoxide production elicited by LTB_4 compared to fMet-Leu-Phe. This second phase in acyl$_2$Gro production also correlated with superoxide production in its sensitivity to cytochalasin B and to changes in extra- or intracellular calcium mobilization.

Cytochalasin B, an actin-binding fungal metabolite, augments many chemoattractant-induced PMN responses, including aggregation, degranulation, and superoxide generation (12). Most recent data demonstrated that acyl$_2$Gro production in response to formyl peptides also were enhanced by this agent (18). In addition, cytochalasin B has been shown to enhance fMet-Leu-Phe-induced intracellular calcium levels and ^{45}Ca uptake and efflux (25–27). A slight enhancement of the initial calcium peak was noted; however, cytochalasin B pretreatment promoted a pronounced second phase of increased cytosolic calcium after exposure to fMet-Leu-Phe but not to LTB_4. The effect of cytochalasin B on cytosolic calcium was abrogated when calcium was removed from the extracellular medium. Similarly, the pronounced second phase of acyl$_2$Gro production elicited by fMet-Leu-Phe in the presence of cytochalasin B was inhibited at least 50% by EGTA, as was the superoxide release under identical conditions. Intracellular buffering of calcium by Quin-2 also caused an even greater reduction in late-phase acyl$_2$Gro elevation, which correlated with inhibition of superoxide release. Notably, neither EGTA nor Quin-2 buffering of calcium affected PtdInsP_2 hydrolysis in human PMNs (28, 29). It therefore appears that cytochalasin B exerts its effect on acyl$_2$Gro levels and superoxide production by augmenting calcium influx.

Our previous results (8, 23) demonstrated that LTB_4 and fMet-Leu-Phe induced equivalent peak levels (5–15 sec) of 1,4,5-InsP_3 and InsP_4. The latter compound has recently been shown to stimulate an extracellular influx of calcium in sea urchin eggs (30). Both 1,4,5-InsP_3 and InsP_4 production were more prolonged in response to fMet-Leu-Phe than to LTB_4, suggesting that the more sustained intracellular calcium concentrations in response to fMet-Leu-Phe might reflect the enhanced production of 1,4,5-InsP_3 and/or InsP_4. However, as cytochalasin B had no effect on the production or metabolism of calcium-mobilizing inositol phosphates, it can be hypothesized that cytochalasin B alters calcium flux by facilitating the ability of second messengers to open calcium permeability channels that have been shown to exist in PMNs (31). The possible role of inositol phosphates in mediating calcium influx has not been reported in PMNs. It has recently been shown that 1,4,5-InsP_3 can be metabolized via two pathways in PMNs. Under ambient calcium concentrations, the products are 1,4-InsP_2, 4-Ins$P$, and inositol. Following simulated PtdInsP_2 hydrolysis and calcium mobilization, the products are InsP_4, 1,3,4-InsP_3, 3,4-InsP_2, 3-Ins$P$, and inositol (23). It will be important to determine whether any inositol metabolite, produced following leukocyte activation, can mediate calcium influx.

The hypothesis that acyl$_2$Gro is produced in response to elevated calcium concentrations has been proposed (32, 33), and indeed acyl$_2$Gro production occurs in response to the calcium ionophore A23187 in HL-60 cells (34). Similar production of acyl$_2$Gro in response to ionomycin occurs in human PMNs (A.P.T. and R.S., unpublished work). That acyl$_2$Gro is being produced and that its degradation is not being inhibited is suggested by the finding of increased phosphatidic acid in PMNs in response to formylpeptide and LTB_4 (9). Notably, production of phosphatidic acid was markedly lower in LTB_4 vs. formylpeptide-stimulated cells, suggesting that differences in the metabolic conversion of acyl$_2$Gro to phosphatidic acid do not account for the relatively poor ability of LTB_4 to induce acyl$_2$Gro.

Although in platelets, elevated intracellular calcium stimulates hydrolysis of phosphatidylinositol by phospholipase C (35, 36), the fatty acid composition of the acyl$_2$Gro formed in fMet-Leu-Phe-stimulated PMNs differs from that of phosphatidylinositol in control cells (37). Additionally our finding that cytochalasin B does not enhance the formation of Ins$P$ in fMet-Leu-Phe-stimulated cells indicates that the enhanced acyl$_2$Gro production does not result from the hydrolysis of phosphatidylinositol. In a previous study that used Dowex columns (38) to quantitate InsP_3, a small enhancement by

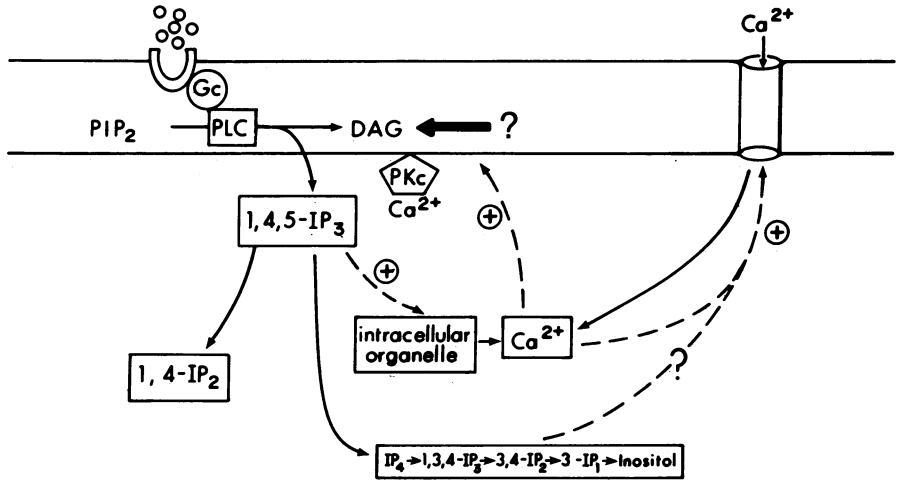

FIG. 5. Model for PMN activation by chemoattractants. Chemoattractant receptor occupancy results in the hydrolysis of PtdInsP_2, which forms 1,4,5-InsP_3, and its metabolites and small amounts of acyl$_2$Gro (DAG). Calcium is initially mobilized from intracellular sources. If sufficiently prolonged, this process leads to an influx of extracellular calcium. The sustained calcium concentration then activates a second source of acyl$_2$Gro necessary for activation of the respiratory burst. The second, major source of acyl$_2$Gro is not derived from inositolphospholipids. Solid lines indicate pathways. Dotted lines indicate stimulated activity. PLC, receptor-coupled phospholipase C; PKc, protein kinase C.

cytochalasin B of fMet-Leu-Phe-stimulated InsP_3 production was found in guinea pig PMNs. However, in the present study, fMet-Leu-Phe plus cytochalasin B caused no significant differences in the levels of the 1,4,5-InsP_3 or 1,3,4-InsP_3 isomers measured by a sensitive and specific HPLC method. We therefore conclude that the increased acyl$_2$Gro seen after 10 sec in fMet-Leu-Phe-stimulated human PMNs is not derived from hydrolysis of inositolphospholipids.

A more complete model of PMN activation can now be proposed (Fig. 5). Chemoattractant receptor occupancy couples a G protein to a phospholipase C that hydrolyzes PtdInsP_2 to form 1,4,5-InsP_3 and acyl$_2$Gro. The 1,4,5-InsP_3 isomer mobilizes calcium from intracellular stores and is metabolized to InsP_4 and, subsequently, 1,3,4-InsP_3, 3,4-InsP_2, 3-Ins$P$, and inositol (23). Products resulting from inositolphospholipid hydrolysis, if sufficiently sustained, may then trigger an influx of calcium contributing to the secondary prolonged rise in intracellular calcium. This later phenomenon is necessary for activation of a distinct pathway for increased acyl$_2$Gro production that stimulates superoxide generation. Our results show that phosphatidylinositol is not the source of this second burst of acyl$_2$Gro production in PMNs. Phosphatidylcholine has recently been identified as a substrate for a phospholipase C (39), and this phospholipid is a potential source of significant amounts of acyl$_2$Gro in other cell types (40), but the second source of acyl$_2$Gro in chemoattractant-stimulated PMNs has not yet been identified. The major difference between fMet-Leu-Phe and LTB$_4$ for activation of the respiratory burst appears to be due to the differential capability of these agents to trigger a sufficiently sustained hydrolysis of PtdInsP_2 (8, 23) to initiate extracellular calcium influx that activates the second acyl$_2$Gro pathway.

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