# A comparative study of the effects of 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine and platelet activating factor on histamine and leukotriene $C_4$ release from human leukocytes

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**Background:** *IgE-mediated stimulation of human basophils and lung mast cells causes the synthesis of larger amounts of 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-acyl-2-acetyl-GPC) than 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor [PAF]).*

**Methods:** *To study the biologic activity of 1-acyl-2-acetyl-GPC, we compared its effects and those of PAF on histamine and leukotriene $C_4$ ($LTC_4$) release from human mixed leukocytes that contained basophils.*

**Results:** *1-Acyl-2-acetyl-GPC (0.1 to 10 μmol/L) failed to release significant amounts of histamine (≥10%) in most donors tested (20 of 24), whereas PAF (0.01 to 1 μmol/L) was active in 58%. 1-Acyl-2-acetyl-GPC (0.1 to 10 μmol/L) was a stimulus for $LTC_4$ release (132 ± 30 ng/μg of histamine) with a potency of about 1000 times less than PAF. The kinetics of 1-acyl-2-acetyl-GPC–activated $LTC_4$ release were similar to those of PAF (half-life ≅ 2 minutes). The specific PAF receptor antagonist, WEB 2086 (10 nmol/L to 10 μmol/L), inhibited both 1-acyl-2-acetyl-GPC- and PAF-mediated $LTC_4$ release with the same potency (inhibitory concentration of 50% ≅ 1.5 μmol/L). Brief (2-minute) cell preincubation with 1-acyl-2-acetyl-GPC in the absence of extracellular $Ca^{2+}$ induced a decrease in the subsequent $Ca^{2+}$ dependent activation of PAF. Similarly, 1-acyl-2-acetyl-GPC (0.1 to 10 μmol/L) caused a concentration-dependent inhibition of PAF-activated histamine secretion (inhibitory concentration of 50% ≅ 0.2 μmol/L).*

**Conclusions:** *Our data suggest that 1-acyl-2-acetyl-GPC may represent, under certain circumstances, a modulator of human basophil mediator release via mechanisms shared with PAF. (J Allergy Clin Immunol 1993;92:325-33.)*

***Key words:*** *Histamine, $LTC_4$, basophils, phospholipids, PAF*

It has long been appreciated that human basophilic leukocytes play an important role in the pathogenesis of allergic diseases through the release of potent mediators such as histamine and leukotriene $C_4$ ($LTC_4$).[1, 2] The activation of human basophils can be accomplished by IgE-dependent or IgE-independent stimuli.[3] Among the latter, the potent lipid mediator platelet activating factor (PAF)[4] has been demonstrated to induce the release of chemical mediators (histamine, $LTC_4$) from human leukocytes.[5, 6] PAF is synthesized by many human inflammatory cells,[7-11] which in turn can be activated by this compound.[7, 8, 12-16]

Recent studies have shown that the activation of a variety of human inflammatory cells leads to the production of both PAF and a compound that is structurally related to PAF, 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-acyl-2-acetyl-GPC).[17-20] 1-Acyl-2-acetyl-GPC differs chemically from PAF in that it contains an ester linkage at the *sn*-1 position. It has been demonstrated that this phospholipid is also different from PAF in

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*Abbreviations used*

| | |
|---|---|
| 1-acyl-2-acetyl-GPC: | 1-Acyl-2-acetyl-*sn*-glycero-3-phosphocholine |
| EDTA: | Ethylenediaminetetraacetic acid |
| HSA: | Human serum albumin |
| $LTC_4$: | Leukotriene $C_4$ |
| PAF: | Platelet activating factor |
| PAG: | PIPES buffer containing 0.003% HSA and 1 gm/L glucose |
| P(10×A)GC: | PIPES buffer containing 0.03% HSA and 1 gm/L glucose + 5 mmol/L $CaCl_2$ |
| PIPES: | Piperazine-N, N'-bis (2-ethanesulfonic acid) |

terms of biologic potency, being far less active (about 1000 times) than PAF.[21-23] However, 1-acyl-2-acetyl-GPC has been shown to inhibit the release of lysosomal enzymes from PAF-activated neutrophils.[23] The present studies were prompted by the surprising finding that after IgE-mediated stimulation, human basophils and lung mast cells generate more 1-acyl-2-acetyl-GPC than PAF.[17, 18] Because the biologic activity of 1-acyl-2-acetyl-GPC has been only partially characterized and because 1-acyl-2-acetyl-GPC is the most abundant "PAF-like" product of the cells involved in the allergic response, we performed experiments to test its biologic function relative to PAF.

## METHODS

### Materials

The following were purchased: piperazine-N, N'-bis (2-ethanesulfonic acid) (PIPES), cytochalasin B, (Sigma Chemical Co., St. Louis, Mo.); crystallized human serum albumin (HSA) (Calbiochem Co., La Jolla, Calif.); $C_{16:0}$ PAF (Biomol, Philadelphia, Pa.); RPMI-1640 (Gibco, Grand Island, N.Y.); dextran T 70 and Percoll (Pharmacia, Piscataway, N.J.); ethylenediamine tetraacetic acid (EDTA), 60% perchloric acid, and dextrose (Fisher Scientific Co., Fair Lawn, N.J.); tritiated $LTC_4$ (New England Nuclear, Boston, Mass.). Goat anti-human IgE was prepared as previously described.[24] 1-Palmitoyl-2-acetyl-glycero-3-phosphocholine was prepared by the addition of acetic anhydride in pyridine to 1-palmitoyl-2-lyso-glycero-3-phosphocholine (Avanti Polar Lipids, Birmingham, Ala.), and its purity was confirmed by negative ion chemical ionization gas chromatography/mass spectrometry of pentafluorobenzyl ester derivatives of PAF and 1-palmitoyl-2-acetyl-glycero-3-phosphocholine. Ions at mass-to-charge ratios of 552 and 566 were monitored for PAF and 1-palmitoyl-glycero-3-phosphocholine, respectively. PAF and 1-acyl-2-acetyl-GPC were resuspended in methanol and stored at −20° C in stock solutions of 1 mg/ml. Solutions of 0.1 mmol/L were prepared just before the experiments by drying aliquots under nitrogen and resuspending them in P(10×A)GC (PIPES buffer containing 0.03% HSA and 1 gm/L glucose + 5 mmol/$CaCl_2$).

$LTC_4$ was a generous gift of Dr. J. Rokach (Merck Frosst, Quebec, Canada); $LTC_4$ antibody was provided by Dr. T. Torphy (SmithKline Beecham, King of Prussia, Pa.); WEB 2086 was donated by Boheringer Ingelheim (Ingelheim, Germany) and BN 52021 by Institut H. Beaufour (Paris, France).

### Buffers

The buffers used in these studies contained 25 mmol/L PIPES, 110 mmol/L NaCl, and 5 mmol/L KCl, pH 7.4. PAG had in addition to 0.1% dextrose and 0.003% HSA. P(10×A)GC, which contained 0.1% dextrose and 0.03% HSA + 5 mmol/L $CaCl_2$, was used during release experiments.

### Preparation of peripheral blood basophils

Venous blood was obtained from normal and atopic volunteers after they had given informed consent. For most experiments, mixed leukocytes that contained basophils (approximately 1%) were prepared as previously described.[25] Briefly, blood was drawn into a final concentration of 0.008 mol/L EDTA and 1.1% dextran and allowed to sediment for 90 minutes at 22° C. The leukocyte-rich upper layer was drawn off; the cells were pelleted (150 *g*, 8 minutes), washed twice with PAG and resuspended in P(10×A)GC for histamine and $LTC_4$ release experiments. We did not perform experiments with purified basophils because we observed previously that purification procedures abolished PAF-induced histamine secretion regardless of the cell purity achieved (M. Columbo, unpublished observations).

### Mediator release

Aliquots that contained approximately 20,000 basophils (i.e., total histamine content of ≅ 20 ng/ml) in 0.1 ml P(10×A)GC were challenged in duplicate with stimuli. The spontaneous release of histamine was assessed by the addition of buffer instead of stimuli and was usually less than 10% of total cellular histamine content. Total histamine content was obtained by lysing the cells with 2% perchloric acid. Replicates differed from each other in histamine content by less than 10%. Results are expressed as percentage of histamine release after subtracting spontaneous release of unstimulated samples from the total histamine content. $LTC_4$ release was expressed as nanograms per microgram of total histamine (human basophils contain approximately 1 pg of histamine per cell). Kinetic experiments were performed by aliquoting cells into tubes that contained different stimuli (e.g., PAF, 1-acyl-2-acetyl-GPC, and buffer control). Aliquots of 0.1 ml were removed in succession at designated time points, mixed

with 0.9 ml 10 mmol/L EDTA at 4° C, and centrifuged (1000 *g*) to obtain cell-free supernatants. In the experiments with 1-acyl-2-acetyl-GPC, concentrations of this lipid higher than 10 μmol/L were not used because of cell toxicity.

### Histamine assay

Histamine release was assayed by the automated fluorometric technique developed by Siraganian.[26]

### $LTC_4$ assay

$LTC_4$ was measured by a radioimmunoassay as described previously[27] with the use of dextran-coated charcoal as the separation technique. The rabbit anti-$LTC_4$ antiserum has been characterized, and its cross-reactivity for heterologous ligands has been described.[27]

### Desensitization experiments

Cells were desensitized by preincubation with stimuli or buffer for 2 minutes at 37° C in P(10×A)G that contained 4 mmol/L EDTA.[28] The cells were then washed twice, resuspended in P(10×A)GC, and challenged with stimuli for 30 minutes for histamine release.

### Statistical analysis

The results are expressed as the means ± SEM of duplicate determinations. Data were analyzed by two-tailed Student's *t* test.

## RESULTS

### Comparison of the effects of 1-acyl-2-acetyl-GPC and PAF on mediator release from human leukocytes containing basophils

Mixed leukocytes from 24 donors were challenged with 1-acyl-2-acetyl-GPC (0.1 to 10 μmol/L) or PAF (1 nmol/L to 1 μmol/L), and the release of histamine was evaluated. In only four donors did we detect significant (≥10%) histamine release with 1-acyl-2-acetyl-GPC (maximal release = 16%), whereas 58% of donors responded to PAF (maximal release = 51%) (Fig. 1, *upper panel*). Because it has been shown that 1-acyl-2-acetyl-GPC is about 1000 times less biologically active than PAF in human neutrophils and platelets,[21, 22] and that cytochalasin B significantly enhances the release of histamine induced by PAF,[6] we challenged the cells of 24 donors with 1-acyl-2-acetyl-GPC (0.1 to 10 μmol/L) and PAF (10 nmol/L to 1 μmol/L) in the presence of cytochalasin B (5 μg/ml).[29] Eighty-three percent of the donors tested released ≥10% histamine after 1-acyl-2-acetyl-GPC stimulation, whereas 100% released significant amounts of histamine to PAF challenge (Fig. 1, *lower panel*). 1-Acyl-2-acetyl-GPC was significantly less potent than PAF in activating histamine release in the presence of cytochalasin B (≅ 1000 times). Because in some donors cytochalasin B itself causes histamine secretion,[6] we also evaluated histamine release with cytochalasin B alone in these experiments and found that it was 5.1% ± 2.2%. As a control for the degree of basophil releasibility, we measured the release of histamine activated by anti-IgE (0.1 μg/ml); this was found to be 45.3% ± 5.5%.

A large amount of earlier work has shown that human leukocytes can release $LTC_4$ on stimulation with anti-IgE, f-met peptide, or calcium ionophore A23187.[2, 3] Because 1-acyl-2-acetyl-GPC is unable to induce significant histamine release in most donors and because PAF is itself a potent stimulus for $LTC_4$ release from human leukocytes that contain basophils,[6] we evaluated the release of $LTC_4$ from our cell preparations challenged with 1-acyl-2-acetyl-GPC (0.1 to 10 μmol/L) and also with PAF (0.001 to 1 μmol/L). The results obtained in these experiments are shown in Fig. 2. 1-Acyl-2-acetyl-GPC and PAF induced the release of large amounts of $LTC_4$ (maximal release = 132 ± 30 and 254 ± 29 ng/μg histamine, respectively, $n$ = 12), and again 1-acyl-2-acetyl-GPC was less potent (≅1000 times) than PAF in inducing $LTC_4$ release. As a comparison, the release of $LTC_4$ activated by anti-IgE (0.1 μg/ml) was 56.9 ± 12 ng/μg histamine. In the same experiments, the release of histamine induced by 10 μmol/L 1-acyl-2-acetyl-GPC was 2.9% ± 1.1%, whereas PAF (1 μmol/L) caused histamine secretion of 13.9% ± 2.4%.

The time course of $LTC_4$ release activated by 1-acyl-2-acetyl-GPC was rapid (half-life ≅ 2 minutes) and similar to that of PAF-induced release (Fig. 3).

### Similarities between the activity of 1-acyl-2-acetyl-GPC and PAF on mediator release from human leukocytes containing basophils

A series of experiments was undertaken to further study the relationship between the activation mechanisms elicited by 1-acyl-2-acetyl-GPC and PAF, and, in addition, to investigate the specificity of these mechanisms. First, cross-desensitization occurred between the two phospholipids. When the cells were preincubated with 1-acyl-2-acetyl-GPC (0.1, 10 μmol/L) or PAF (0.01, 1 μmol/L) for 2 minutes in the absence of extracellular $Ca^{2+}$ and then washed, there was

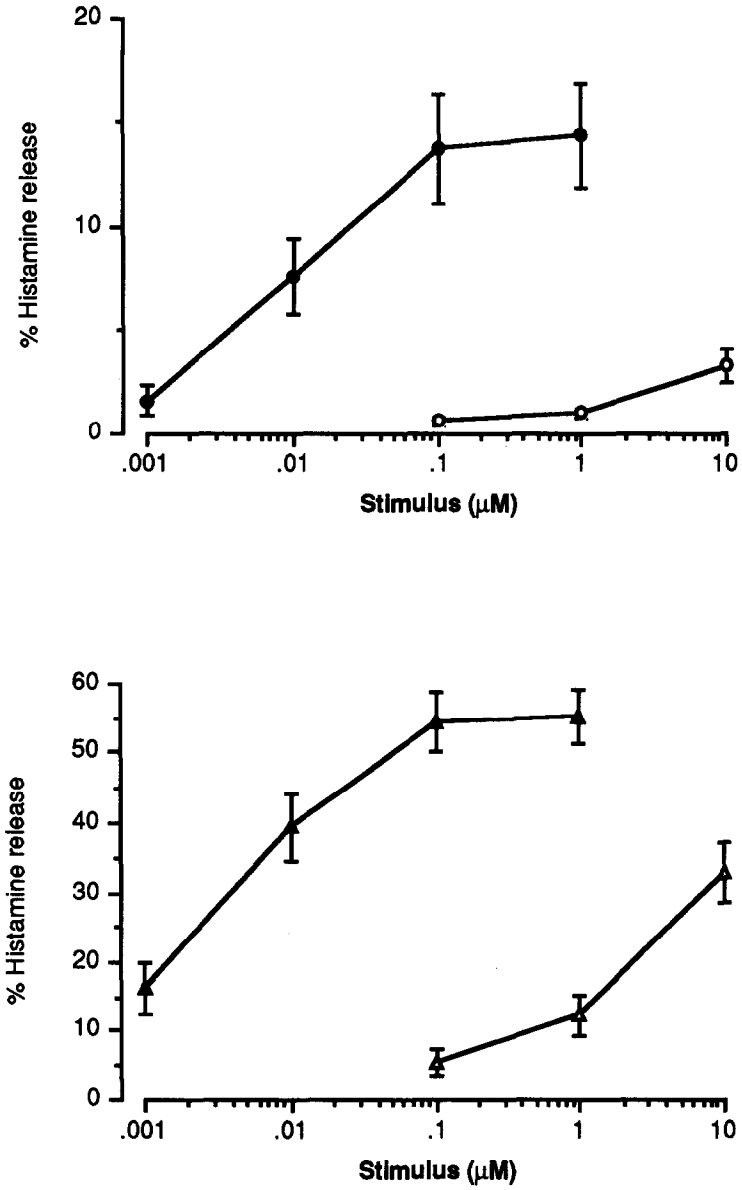


**FIG. 1.** The effect of increasing concentrations of 1-acyl-2-acetyl-GPC *(acyl, open symbols)* or PAF *(closed symbols)* in the absence (*circles,* **upper panel**) or in the presence of (*triangles,* **lower panel**) of 5 μg/ml cytochalasin B on histamine release from human basophils. The cells were challenged for 30 minutes at 37° C. Each point represents the mean ± SEM of the results obtained in 24 different donors.

decreased histamine release to a subsequent PAF (1 μmol/L) challenge in the presence of $Ca^{2+}$ when compared with untreated controls (Table I). Similar results were obtained when a lower concentration of PAF (0.1 μmol/L) was used to induce histamine release (data not shown). We also examined the effect of the specific PAF receptor antagonist, WEB 2086[30] (10 nmol/L to 10 μmol/L), on PAF (1 μmol/L) and 1-acyl-2-acetyl-GPC (10 μmol/L)-induced $LTC_4$ release (Fig. 4). The inhibition curves and the inhibitory concentration of 50% (1.5 μmol/L) obtained for both stimuli indicate that 1-acyl-2-acetyl-GPC and PAF initiate $LTC_4$ release from human leukocytes by acting on a common receptor. These data were further confirmed with the use of another PAF-specific receptor antagonist, compound BN 52021.[31] BN 52021 caused equivalent inhibition of 1-acyl-2-acetyl-GPC- and PAF-induced $LTC_4$ release (data not shown).

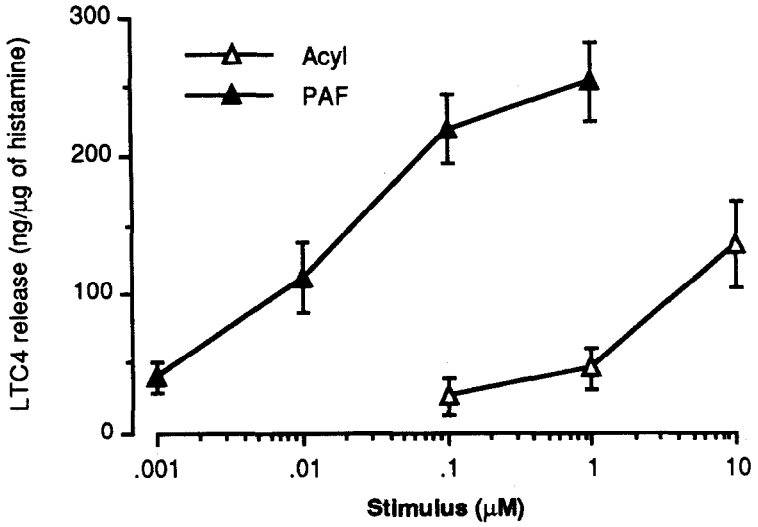


**FIG. 2.** The effect of increasing concentrations of 1-acyl-2-acetyl-GPC *(acyl, open symbols)* or PAF *(closed symbols)* on $LTC_4$ release from human mixed leukocytes. The cells were challenged for 30 minutes at 37° C. Each point represents the mean ± SEM of 12 experiments performed with different donors.

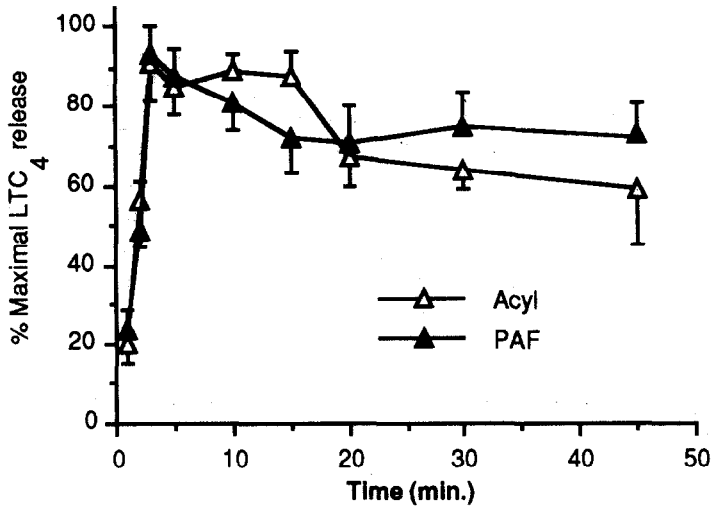


**FIG. 3.** Time course of the effect of 10 μmol/L 1-acyl-2-acetyl-GPC *(acyl)* and 1 μM PAF on the release of $LTC_4$ from human mixed leukocytes. Each point represents the mean ± SEM of three experiments performed with different donors.

## Modulation of PAF-induced human basophil histamine release by 1-acyl-2-acetyl-GPC

To ascertain whether 1-acyl-2-acetyl-GPC, which did not cause significant histamine release in most donors, could affect PAF-induced histamine release, we pretreated mixed leukocytes with 1-acyl-2-acetyl-GPC (0.1, 10 μmol/L) and then challenged the cells with PAF (10 nmol/L to 1 μmol/L). Fig. 5, *A* shows that 1-acyl-2-acetyl-GPC induces a potent inhibition of histamine release activated by PAF. This inhibitory effect was still present when we used a concentration of 1-acyl-2-acetyl-GPC 10 times lower than PAF. Very similar inhibition was obtained in another series of experiments in which cytochalasin B (5 μg/ml) was used together with PAF to induce histamine secretion ($n$ = 9, data not shown). No inhibitory effect by 1-acyl-2-acetyl-GPC was found when anti-IgE was used as a stimulus for histamine release (Fig. 5, *B*). The kinetics of 1-acyl-2-acetyl-GPC–induced inhibition of the release of histamine activated by PAF were rapid, being

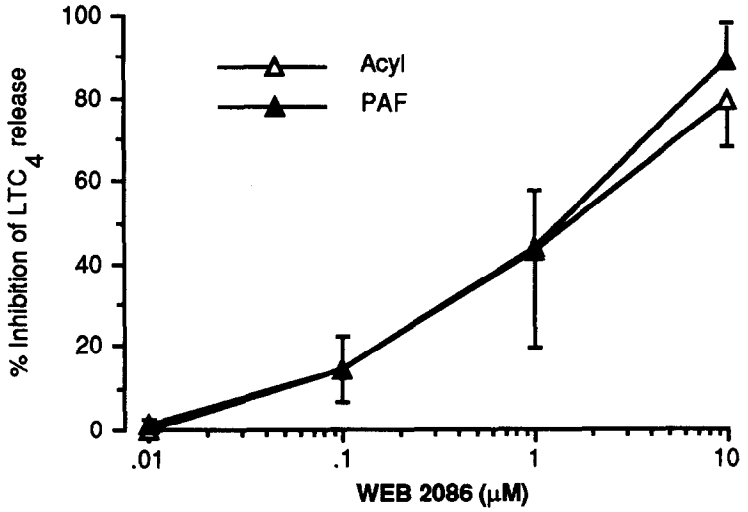


**FIG. 4.** Effect of increasing concentrations of the PAF receptor antagonist WEB 2086 on the release of $LTC_4$ induced by 1-acyl-2-acetyl-GPC (*acyl*, 10 μM) or PAF (1 μM). The cells were preincubated with WEB 2086 for 3 minutes before the addition of the stimuli and then incubated for an additional 30 minutes. The control $LTC_4$ release induced by 1-acyl-2-acetyl-GPC and PAF was 60.7 ± 11 ng/μg of histamine and 218 ± 53 ng/μg of histamine, respectively. Each point represents the mean ± SEM of three experiments performed with different donors.

**TABLE I.** Desensitizing effect of PAF and 1-acyl-2-acetyl-GPC on PAF-induced histamine release from human basophils

| | Desensitizing agent | | | | |
|---|---|---|---|---|---|
| Experiment | None | PAF (0.01 μmol/L) | PAF (1 μmol/L) | Acyl (0.1 μmol/L) | Acyl (10 μmol/L) |
| | | | % Histamine release | | |
| 1 | 39 | 23 | 3 | 33 | 21 |
| 2 | 19 | 14 | 0 | 16 | 13 |
| 3 | 13 | 8 | 0 | 11 | 5 |

The experiments were performed with different donors. See Methods section for details on the procedure.
*Acyl*, 1-Acyl-2-acetyl-GPC.

maximal after 2 minutes of cell preincubation with the compound before the challenge with PAF (data not shown).

## DISCUSSION

It has been shown that on stimulation, a variety of human cells such as neutrophils, platelets, and lung mast cells synthesize not only PAF but another 2-acetylated phospholipid, 1-acyl-2-acetyl-GPC.[17-20] In cells such as the neutrophil, eosinophil, and macrophage the amount of 1-acyl-2-acetyl-GPC produced is quantitatively less than PAF,[18] but it has been demonstrated recently that IgE-mediated activation of human basophils and lung mast cells yields a larger amount of 1-acyl-2-acetyl-GPC than PAF.[17, 18]

The results of the studies presented here indicate that the naturally occurring phospholipid, 1-acyl-2-acetyl-GPC, is able to activate significant (>10%) histamine secretion in human basophils in only a minority of donors, whereas PAF is active in the majority. Coincubation of 1-acyl-2-acetyl-GPC with cytochalasin B[29] led to significant histamine release in most donors tested. The comparison between the release of histamine obtained with 1-acyl-2-acetyl-GPC and PAF in the presence of cytochalasin B suggests that 1-acyl-2-acetyl-GPC is about 1000 times less active than PAF in causing histamine secretion from human basophils. These results are in agreement with previous studies showing that 1-acyl-2-acetyl-GPC is about 1000 times less active than PAF in

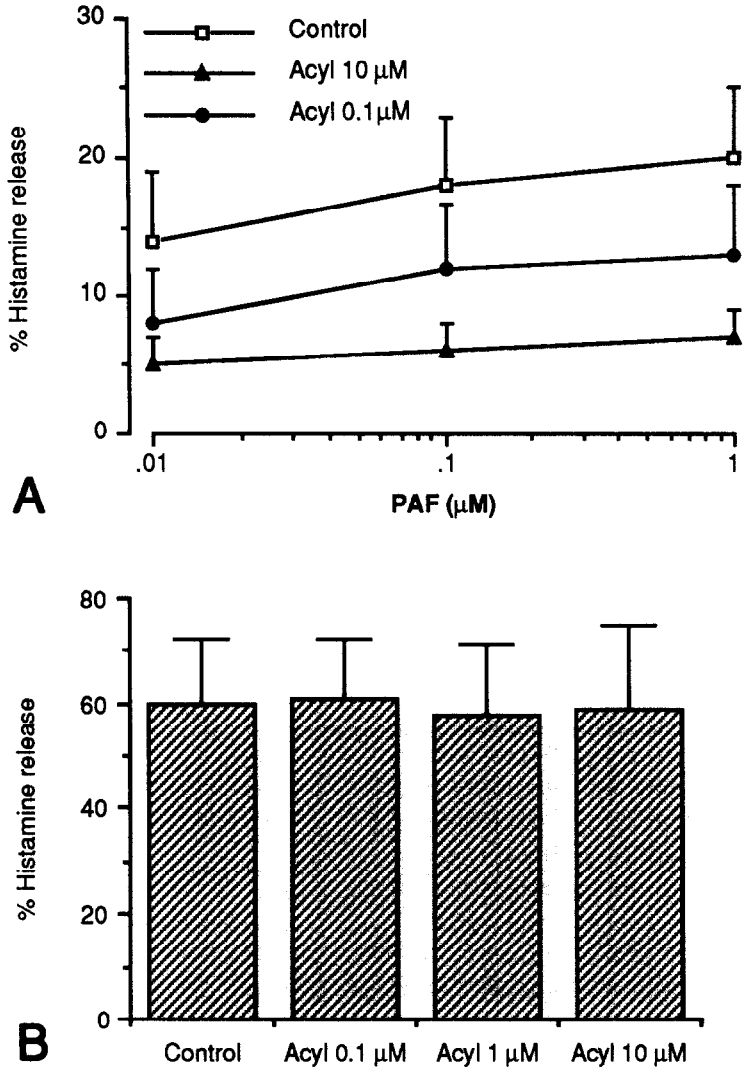


**FIG. 5. A,** Effect of 1-acyl-2-acetyl-GPC *(acyl)* (0.1, 10 μM) on the release of histamine induced by three different concentrations of PAF. The cells were preincubated with 1-acyl-2-acetyl-GPC for 3 minutes and then challenged for PAF for an additional 30 minutes. Each point represents the mean ± SEM of three experiments performed with different donors. **B,** Effect of increasing concentrations of 1-acyl-2-acetyl-GPC *(acyl)* on the release of histamine induced by anti-IgE (0.1 μg/ml) (range of histamine release = 41% to 82%). The cells were preincubated with 1-acyl-2-acetyl-GPC for 3 minutes and then challenged with anti-IgE for an additional 30 minutes. Each point represents the mean ± SEM of three experiments performed with different donors.

neutrophils and platelets[21, 22] and that cytochalasin B can exert a potent enhancing effect on histamine release from human basophils induced by lipid compounds.[6, 32]

We have found that PAF activates the release of large amounts of $LTC_4$ from human mixed leukocytes.[6] Our present results indicate that 1-acyl-2-acetyl-GPC is also a stimulus for $LTC_4$ release from human mixed leukocytes that contain basophils. Platelets, monocytes, and eosinophils, which were present in varying percentages in our cell preparations, are also able to release $LTC_4$ on challenge with different stimuli. On the basis of the relative number of these cells and their ability to produce mediators,[13, 33-35] platelets, monocytes, and eosinophils do not appear to be likely candidates for a pivotal role in 1-acyl-2-acetyl-GPC– and PAF-induced $LTC_4$ release. However, we can only speculate that the majority of the $LTC_4$ released by PAF-stimulated mixed leukocytes is the product of basophils, because a cooperative effect of different cell populations cannot be ruled out.

The similarity between the pathways that lead

to $LTC_4$ release by 1-acyl-2-acetyl-GPC and PAF is directly supported by several lines of investigation. First, the similarity between the kinetics of the release of $LTC_4$ induced by two phospholipids suggests that the mechanisms are similar. Second, the cross-densensitization results support the hypothesis that 1-acyl-2-acetyl-GPC and PAF activate histamine and $LTC_4$ release from human mixed leukocytes via the same pathway. Finally, the studies with the specific PAF receptor antagonist, WEB 2086,[30] which inhibits 1-acyl-2-acetyl-GPC- and PAF-induced $LTC_4$ release with the same potency, suggest that the two phospholipids probably act on a common, specific membrane receptor. It is worth noting that cross-desensitization experiments also indicate that the inhibitory effect of 1-acyl-2-acetyl-GPC on PAF-induced histamine release is still present after cell washing and does not depend on the presence of extracellular $Ca^{2+}$.

Recent studies have shown that 1-acyl-2-acetyl-GPC is able to inhibit the release of lysosomal enzymes from human neutrophils stimulated by PAF.[23] Our studies demonstrated that 1-acyl-2-acetyl-GPC exerted a potent inhibitory effect of PAF-induced histamine secretion. The inhibition appeared to be noncompetitive in that it was not surmounted by increasing the concentration of PAF. Because it has been recently demonstrated that the immunologic activation of human inflammatory cells such as basophils and lung mast cells yields a greater amount of 1-acyl-2-acetyl-GPC than PAF,[22, 23] our data suggest that 1-acyl-2-acetyl-GPC may represent an endogenous inhibitor of human basophil histamine release caused by PAF.

In summary, our experiments have characterized the activity of the naturally occurring phospholipid, 1-acyl-2-acetyl-GPC, on mediator release from human leukocytes containing basophils and compared its activity to PAF. It appears that 1-acyl-2-acetyl-GPC possesses the ability to act as both an agonist ($LTC_4$ release) and an antagonist (histamine release) of PAF without having in most cases significant histamine-releasing activity in the absence of cytochalasin B. It is also evident that 1-acyl-2-acetyl-GPC acts on leukocytes through activation steps that are shared to a great extent with PAF. Although the mechanisms by which 1-acyl-2-acetyl-GPC exerts its effects on the human basophils, as well as other cell types,[23] have not been completely clarified, our studies suggest that this molecule, which is produced in large amounts by immunologically stimulated mast cells and basophils, may play a modulatory role in the allergic response.

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