

THE JOURNAL OF Allergy AND Clinical Immunology

July 1994 • Volume 94 • Number 1

Excretion of platelet activating factor–acetylhydrolase and phospholipase A₂ into nasal fluids after allergenic challenge: Possible role in the regulation of platelet activating factor release

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Platelet activating factor (PAF), a proinflammatory mediator synthesized through a phospholipase A₂ (PLA₂)-dependent reaction, is hydrolyzed into its inactive metabolite, lyso-PAF, by a specific acetylhydrolase. Previous studies have shown that allergen challenge of patients with allergic rhinitis leads to an increase of the concentrations of lyso-PAF in nasal lavage fluid (NLF), whereas PAF is detected only marginally. PAF-hydrolyzing enzymes are expected to be released on allergenic challenge, to account for the reduced concentrations of PAF in NLF. Here, we show that allergen challenge of patients with allergic rhinitis induces an increase of acetylhydrolase-like activity in NLF, which peaks within 10 minutes and returns to basal values 1 hour later. Acetylhydrolase hydrolyzed exogenous PAF with a complete loss of its ability to induce platelet aggregation. Allergen challenge also led to a parallel release of a PLA₂ in nasal fluids. This enzyme preferentially hydrolyzes negatively charged phospholipids (phosphatidic acid monomethyl ester and phosphatidylglycerol) versus phosphatidylcholine. More interestingly, the hydrolysis of phosphatidic acid monomethyl ester and phosphatidylglycerol by NLF was completely abolished by the addition of ethylenediaminetetraacetic acid which had no effect on the hydrolysis of PAF, indicating that the PLA₂ secreted in nasal fluids is not involved in the degradation of PAF. Finally, our results show that allergen-induced increase in the concentrations of lyso-PAF and prostaglandin D₂ occurred with a kinetic similar to that of tosyl-L-arginine-methyl-ester esterase, suggesting that mast cells are implicated in this process. Although no direct relationship was demonstrated between the absence of PAF and the increase of acetylhydrolase levels in NLF, we suggest a potential role for this enzyme in the inactivation of PAF if the latter is released in the nasal lumen. (J ALLERGY CLIN IMMUNOL 1994;94:109-19.)

BSA	Bovine serum albumin
EDTA	Ethylenediaminetetraacetic acid
FEV₁	Forced expiratory volume in 1 second
NAR	Nasal airway resistance
NLF	Nasal lavage fluid
PA	Phosphatidic acid monomethyl ester
PAF	Platelet activating factor
PC	Phosphatidylcholine
PG	Phosphatidylglycerol
PGD₂	Prostaglandin D ₂
PLA₂	Phospholipase A ₂
TAME	Tosyl-L-arginine methyl ester

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Key words: [Allergic rhinitis](#), [PAF](#), [lyso-PAF](#), [acetylhydrolase](#), [phospholipase A₂](#)

Platelet-activating factor (PAF)¹ stimulates inflammatory cells and induces symptoms of allergic reactions such as vasodilatation, hypotension, increased vascular permeability mucus secretion, leukocyte migration, and, although indirectly, smooth muscle contraction.^{2 3} PAF is synthesized through a phospholipase A₂ (PLA₂)-dependent reaction and hydrolyzed into its inactive metabolite, lyso-PAF, by a specific acetylhydrolase.^{2 3} Because PAF is produced by a number of cells and organs at concentrations above those required for its biologic activities, it may play a critical role in inflammation and allergic reactions.^{2 3} Nasal challenge with allergen induces sneezing, obstruction, and mucus hypersecretion, accompanied by increased levels of histamine, tosyl-L-arginine-methyl-ester (TAME) esterase, kinins, sulfidopeptide leukotrienes, prostaglandin D₂ (PGD₂) in the nasal lavage fluid (NLF).^{4 7} In these conditions PAF was detected marginally, whereas the concentrations of its metabolite lyso-PAF increased markedly after nasal challenge.^{8 9} PAF may not be excreted, or a PAF-hydrolyzing enzyme may be released on allergenic challenge, to account for its reduced concentrations in the nasal lumen. Acetylhydrolase is involved in the hydrolysis of PAF into its inactive metabolite lyso-PAF.^{10 11} The levels of acetylhydrolase in plasma may be critical for the regulation of the amounts of circulating PAF and, accordingly, of its biologic activities.² Indeed, low levels of plasma acetylhydrolase have been associated with the severity of respiratory symptoms in children with asthma, potentially because of an excess of circulating PAF.¹² The aim of this study was to evaluate the presence of PAF-hydrolyzing enzymes in the nasal secretions and the effect of allergen provocation on their secretions. The possible role of such enzymes in the regulation of PAF concentrations in the nasal lumen is discussed.

Methods

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Subjects

Ten adults (mean age, 24 ± 1.8 years), selected on the basis of a well documented history of seasonal allergic rhinitis were studied after informed consent was obtained. All were free of symptoms (out of season) but had a verified hypersensitivity to grass pollen—positive skin prick and nasal challenge tests. The trial was conducted over a pollen-free period of 3 months (December to February).

Nasal challenges and assessment of response

The nasal challenges with grass pollen (*Dactylis glomerata*; Laboratoires Stallergènes, Fresnes, France) were performed with a nasal pump spray delivering 100 µl per spray in each nostril, as previously described.⁸ The challenge solutions were kept at room temperature. As the subjects voluntarily suspended respiration, the challenge material was sprayed onto the inferior turbinate area of both nostrils. After each spray, the solution was kept in contact with the nasal mucosa for 10 minutes with a nose clip, which was removed 2 minutes before nasal airway resistance (NAR) was measured by active posterior rhinomanometry (Rhinomat; Electronique Medical Informatique,

France). Forced expiratory volume in 1 second (FEV₁) was measured before and after the challenge (Autospiro-AS-500; Minato, Osaka, Japan).

FEV₁ and NAR were measured 1 hour before the allergen challenge (T = -60 minutes). Then, at T = -20 minutes, the patients were challenged with saline solution, followed 10 minutes later by the evaluation of FEV₁ and NAR to establish the nasal clinical scores. Three lavages were then carried out; the last one was retained for the measurement of mediator and enzyme release. At T = 0 minutes, the patients were challenged with two sprays in each nostril of 0.1 ml of allergen extract at 1 reactivity index/ml (i.e., 0.1 reactivity index/nostril). The NAR was measured and the clinical scores were established at T = +10 minutes, followed by a lavage. These operations were repeated at T = +20, +30, +60, +120, and +180 minutes. The clinical symptoms (blockade, secretion, and number of sneezes) recorded before and 15 minutes after the nasal challenge were scored as absent (0), mild (1), moderate (2), and severe (3). From these recordings, a composite nasal score was calculated as the sum of the individual scores. The test was positive for a score greater than 5. Aliquots (1 ml) from NLF were collected at the 10- or 20-minute intervals with Eppendorf tubes (Eppendorf, Hamburg, Germany) and immediately centrifuged for 2 minutes at 12,000 rpm to remove the contaminating cells. The supernatants were stored at -20° C until use.

Measurement of acetylhydrolase activity

After thawing, the acetylhydrolase activity of NLF was measured as described by Blank et al.¹⁰ Briefly, 2 aliquots (0.5 ml) from each tube were incubated with 0.05 µCi of radioactive PAF, 1-O-octadecyl[2-³H]-acetyl-sn-glycero-3-phosphocholine (CEA, Saclay, France) and mixed with unlabeled PAF to a final concentration of 10 µmol/L. Both labeled and unlabeled PAF were first resuspended in saline solution containing 1.25% fatty acid-free bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.) before incubation with NLF. The tubes were vortexed for 30 seconds and incubated for 10 minutes at 37° C. In certain experiments 5 mmol/L ethylenediaminetetraacetic acid (EDTA) or 5 mmol/L CaCl₂ was added to the tubes immediately before PAF. The reactions were then terminated with 1 ml chloroform/methanol (1:1 vol/vol), and phase partition was promoted by 2-minute centrifugation at 12,000 rpm. Samples from the aqueous phases containing free tritiated acetate were carefully aspirated, and their radioactive contents were measured by liquid scintillation counting. Hydrolysis of PAF was verified by submitting organic phases to thin-layer chromatography as previously described.¹³ This showed that the increase of radioactive acetate in the aqueous phase closely correlated to the decrease of radioactive PAF in the organic phase. No significant radioactivity was observed in the aqueous phase when radioactive PAF was incubated with distilled water, indicating that no spontaneous release of acetate occurred under these conditions. Furthermore, the recovery of acetate into the aqueous phase, assessed with radioactive acetate as internal standard, was greater than 95%. The acetylhydrolase activity in each tube was evaluated in picomoles of PAF hydrolyzed per minute in 1 ml of NLF and represents the mean of duplicate measurements. The limit of detection of acetylhydrolase activity by this method was 1 pmol/ml/min.

PLA₂ fluorometric assay

Fluorometric assay

The PLA₂ assay was carried out by the fluorometric method described by Radvanyi et al.¹⁴ Routine enzyme assay used the fluorescent phospholipid substrate 1-palmitoyl-2-(10-pyrenyldecanoyl)-sn-glycero-3-monomethyl phosphatidic acid (monomethyl-PA; Interchim, Montluçon, France). In certain experiments 1-palmitoyl-2-(10-pyrenyldecanoyl)-sn-glycero-3-phosphocholine (PC) and 1-palmitoyl-2-(10 pyrenyldecanoyl)-sn-glycero-3-phosphatidylglycerol(PG) (Interchim) were also used as substrates. These phospholipids were dried under nitrogen and suspended in ethanol at 0.2 mmol/L. Vesicles of fluorescent phospholipid were prepared as previously reported.¹⁴ The reaction solution was prepared by introducing sequentially, in a plastic cuvette, 980 µl of buffer containing 50 mmol/L Tris-HCl, 100 mmol/L NaCl, and 1 mmol/L ethyleneglycol-bis-(β-aminoethylether)-N,N,N•, N•-tetraacetic acid (pH 7.5); 10 µl of substrate (2 µmol/L, final solution) and 10 µl of a 10% fatty acid-free BSA solution (0.1%, final solution). Aliquots (10 to 50 µl) from NLF were added and allowed to equilibrate at 37° C for 1 minute. The reactions were then initiated by the addition of 10 µl of CaCl₂ (1 mol/L).

Fluorescence was determined with a xenon lamp, and all the reactions were carried out in 4 × 10 mm disposable plastic cuvettes. The fluorescence intensity was monitored with excitation and emission wavelengths of 345 and 398 nm, respectively, with a slit width of 4 nm. The limit of detection of PLA₂ activity was approximately 0.5 nmol hydrolyzed phospholipid per milliliter per minute.

Hydrolysis of 1-palmitoyl-2-[¹⁴C]-arachidonoyl-sn-glycero-3-phosphorylcholine

This assay was carried out essentially as described by Kramer et al.¹⁵ with 1-palmitoyl-2-[¹⁴C]-arachidonoyl-sn-glycero-3-phosphoryl choline (2-[1-¹⁴C]arachidonoyl-PC) as substrate. The incubation buffer was composed of 100 mmol/L Tris-HCl (pH 9), 5 mmol/L CaCl₂, 0.5 mg/ml fatty acid-free BSA, and 1 mmol/L 2-mercaptoethanol. The substrate consisted of 6 nmol of radiolabeled phospholipid and 4 nmol of diacylglycerol vesicles prepared by sonication in 1 ml of 100 mmol/L Tris buffer (45 seconds twice at 150 W in an ice bath). Kramer et al.¹⁵ have demonstrated that the diacylglycerol incorporated into the phosphatidylcholine substrate stimulates the PLA₂ activity by approximately fourfold and that the optimum pH for this activity is 9. The reaction was started by adding 100 µl of NLF or 0.4 µg of PLA₂ from *Crotalus durissus terrificus* as positive control for hydrolysis in a final volume of 300 µl. The reaction was performed at 37° C for 30 minutes and stopped by the addition of chloroform/methanol (1:2, vol/vol). Lipid extraction was carried out according to the method of Bligh and Dyer,¹⁶ then the organic phases were evaporated to dryness and the lipids redissolved with 50 µl of chloroform/methanol (2:1). The phospholipids were separated in thin-layer chromatography with chloroform/methanol/acetic acid/water (65:43:1:3) as a mobile phase. Authentic unlabeled phospholipids were spotted on the same plates as standards and visualized by iodine vapors. The plates were then divided into 17 bands, which were scraped off in order to measure their radioactivity content by liquid scintillation spectrophotometry.

PAF and lyso-PAF assays

Aliquots (1 ml) of NLF were immediately recovered in glass tubes containing 2 ml of cold methanol, to prevent the hydrolysis of PAF, and then vortexed and stored at -20° C until use. Lipid extraction

was carried out according to the method of Bligh and Dyer,¹⁶ and the organic phases were removed and evaporated to dryness under oxygen-free nitrogen. Lipids were then redissolved in 50 μ l of chloroform/methanol (2:1) and separated by thin-layer chromatography on silica gel Si 60 plates (Merck, Darmstadt, Germany) with chloroform/methanol/ammonia (70:35:7) as mobile phase.¹³ The areas containing PAF and lyso-PAF were identified by comparison with the corresponding synthetic standards (Sigma Chemical Co.) added to the same plate and then extracted as indicated above. The lyso-PAF fractions were converted to PAF by chemical acetylation as described by Benveniste et al.¹⁷ The yields of this method of separation vary from 75% to 85% for PAF and from 55% to 75% for lyso-PAF, and these yields were taken in account to quantify these compounds. After evaporation, the extracts were redissolved in 250 μ l of saline solution containing 0.25% BSA and kept at -4° C. Then, the amounts of PAF and acetylated lyso-PAF were determined with aggregation of washed aspirin-treated rabbit platelets¹⁷ on the basis of standard curves and expressed as equivalent amounts of synthetic PAF. This method allows detection of PAF and lyso-PAF in NLF at concentrations as low as 1 ng/ml. The authenticity of PAF was controlled by 2-minute preincubation of platelets with 50 μ mol/L of the specific PAF antagonist WEB 2086 (Boehringer Mannheim GmbH, Mannheim Germany).

TAME esterase activity

The esterase activity was measured by the radiochemical assay of Beaven et al.,¹⁸ based on the liberation of tritium-labeled methanol from the synthetic substrate p-tosyl-L-arginine [³H]methyl-ester ([³H]TAME, 1 Ci/mmol, Amersham). Tritiated TAME is hydrolyzed to yield tritiated methanol, which is separated from unhydrolyzed tritiated TAME by a column of carboxylin acid ion exchange resin (Biorex; Bio-Rad, Ivry, France), which retains the tritiated TAME, whereas tritiated methanol passes through and is measured in the effluent by liquid scintillation spectrophotometry.

PGD₂

The amounts of PGD₂ in NLF were measured with a competitive immunoenzymatic method, as previously described.¹⁹ The proteins were precipitated by acetone/ethanol (70:30, vol/vol) and PGD₂ converted to PGD₂-Methoxin (PGD₂-Mo) with methoxamine hydrochloride dissolved in pyridine. The concentration of PGD₂-Mo was determined by a spectrophotometric measurement at 420 nm (Multiskan Labsystem, France), with the detection limit being 30 pg/ml of biologic fluids.

Protein assay

Aliquots of NLF were centrifuged for 10 minutes at 1000g, and the supernatants were collected for protein assay as described by Sano Shiba et al.²⁰

Analysis of data

All data are reported as means $\pm$ SEM of individual determinations. For each patient, the measurements are carried out in duplicate. Wilcoxon's test was used for the analysis of the effect of

allergen challenge and *p* values are significant at less than 0.05, less than 0.01, and less than 0.005.

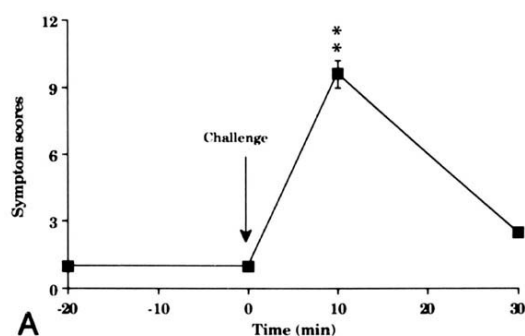
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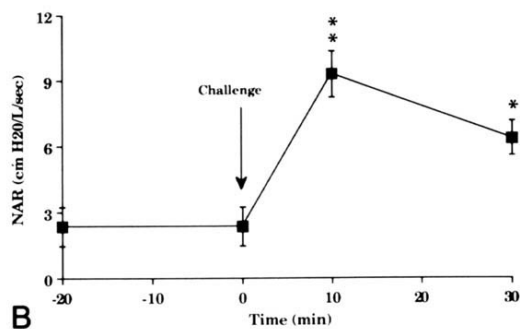
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Clinical symptoms

Repeated nasal lavages with saline solution, carried out before the provocation with allergen, had no significant effect on symptom scores or on NAR (■ Fig. 1). The final values of NAR, obtained after the fourth lavage, did not differ significantly from the baseline value, obtained before the first lavage. As reported,^{4,8} repeated lavages with saline solution reduced the basal concentrations of proteins in NLF (data not shown). Nasal provocation with grass pollen induced an early response with sneezing, rhinorrhea, and nasal obstruction, thus confirming the findings of previous studies.⁴⁻⁹ Ten minutes after allergen administration, the symptom score increased dramatically (from 0.25 ± 0.46 to 9.00 ± 3.21 , $p < 0.005$) with a concomitant increase in the NAR (from 2.81 ± 0.28 to 8.07 ± 2.67 cm H₂O/L/sec, $p < 0.005$). Both symptom scores and NAR returned nearly to their basal values 30 minutes after the challenge (2.75 ± 2.05 and 3.47 ± 1.08 cm H₂O/L/sec, respectively) (■ Fig. 1).

FIG. 1. Changes in symptom scores (A) and NAR (B) after allergen challenge of patients with allergic rhinitis (means $\pm$ SEM, $n = 10$). Nasal challenges with saline solution or allergen were carried out at T = -10 minutes and T = 0 minutes, respectively. Symptom scores and NAR were measured 20 minutes before (-20 minutes), immediately before (0 minutes), 10 minutes, and 30 minutes after the challenge. The arrows indicate the moment when saline solution (control) or allergen was applied to the patients.





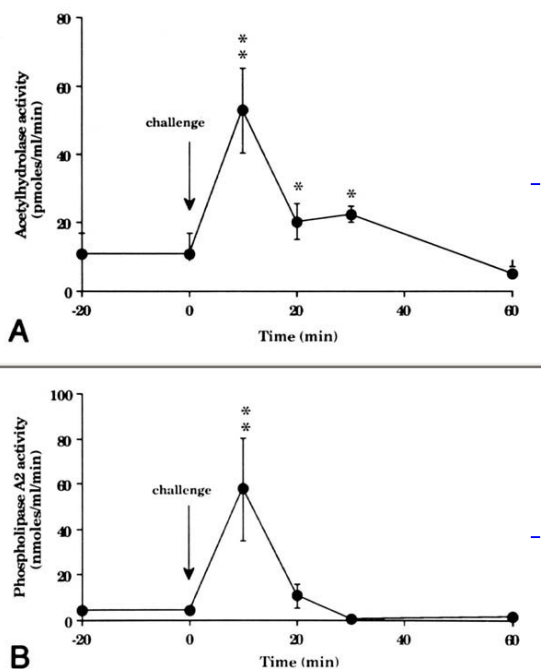
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During the course of the study, no significant change in the FEV₁ was observed. FEV₁ was evaluated at 4.24 ± 0.43 and 4.20 ± 0.41 before and 30 minutes after allergen challenge.

Excretion of acetylhydrolase, PLA₂ and TAME esterase

Preliminary experiments were carried out to determine the kinetic parameters of PAF hydrolysis in NLF. These experiments showed that this reaction was linear up to a 30-minute incubation of NLF with 10 $\mu\text{mol/L}$ of exogenous PAF (data not shown). Then, the routine enzyme assay of acetylhydrolase activity was performed by incubating NLF with 10 $\mu\text{mol/L}$ of radioactive PAF for 10 minutes at 37° C. ■ Fig. 2, A shows that NLF, collected before allergen challenge, contained a basal acetylhydrolase activity (10.9 ± 6.2 , pmol hydrolyzed PAF/ml/min; $n = 10$). The allergen provocation induced a significant increase of this activity, which reached its maximal level (52.8 ± 12.4 pmol/ml/min, $p < 0.01$, $n = 10$) 10 minutes after the provocation. The effect of allergen on the increase of acetylhydrolase activity in NLF remained significant up to 30 minutes after the challenge and then returned to basal values within 1 hour (■ Fig. 2, A).

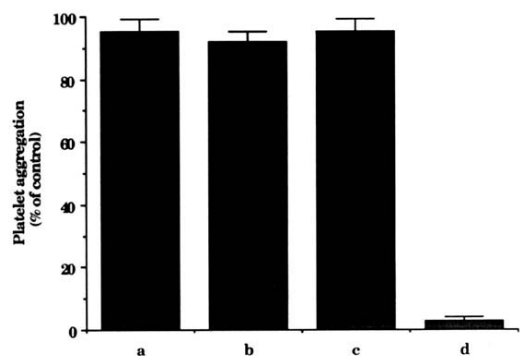
FIG. 2. Time course of the secretion of acetylhydrolase (A) and PLA₂ (B) into NLF after allergen challenge. The allergen was applied to the patients, and then NLF samples were collected at the time points indicated. Enzyme activities were determined as detailed in the Methods section. The results are the means $\pm$ SEM of individual values ($n = 10$). The arrows indicate the moment when allergen was applied to the patients. In control experiments saline solution was applied to the patients 20 minutes before allergen challenge and had no significant effect on the levels of the enzymes. * $p < 0.05$, ** $p < 0.01$, versus control (0 minutes) before the challenge.



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Another approach was used to investigate the capacity of nasal fluids to inactivate PAF. Unlabeled PAF (10 nmol/L) was incubated for 1 hour at 37° C or at 4° C with NLF collected 10 minutes after the challenge. After lipid extraction, these NLF samples were tested for their ability to induce platelet aggregation. The results showed that the incubation at 37° C led to a complete disappearance of the PAF-aggregating activity, which remained unchanged in the tubes incubated at 4° C (■ Fig. 3).

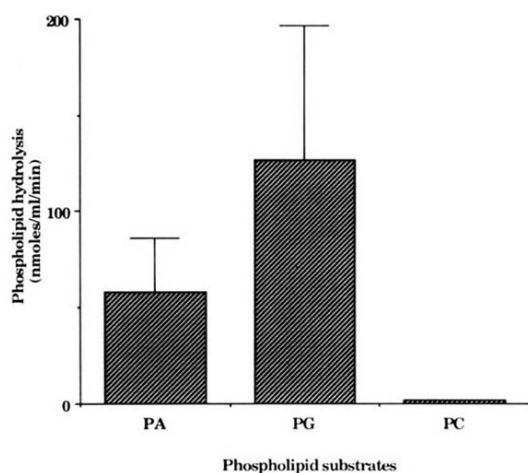
FIG. 3. Effect of NLF on the ability of PAF to induce platelet aggregation. Unlabeled PAF (10 nmol/L, final) was incubated with 0.25 ml of NLF for 1 minute or 1 hour at 37° C or at 4° C. The reactions were stopped, and lipid was extracted as indicated in the Methods section. Then, the extracts were tested for their ability to induce the aggregation of rabbit platelets. The results show the extent of aggregation induced by each extract and expressed as percent (mean $\pm$ SEM) of control in which PAF was incubated with saline solution. *a*, NLF + PAF, 1 min (4° C); *b*, NLF + PAF, 1 hour (4° C); *c*, NLF + PAF, 1 minute (37° C); *d*, NLF + PAF, 1 hour (37° C).



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NLF also contained a PLA₂ activity with levels that increased from basal levels of 11.5 ± 2.8 nmol/ml/min to a maximal level of 57.8 ± 22.2 nmol/ml/min of hydrolyzed PA, 10 minutes after the provocation, and excreted with a kinetic similar to that of acetylhydrolase (■ Fig. 2, B). The PLA₂ activity released in nasal fluids had an apparent specificity for negatively charged phospholipids (PA and PG) versus PC (■ Fig. 4). More interestingly, the hydrolysis of PA and PG by NLF was completely abolished by the addition of EDTA, which had no effect on the hydrolysis of PAF (■ Table I).

FIG. 4. Effect of NLF on the hydrolysis of PA, PG, and PC. Aliquots (10 to 50 μ l) from NLF collected 10 minutes after the challenge were introduced into the cuvettes containing PA, PG, and PC (2 μ mol/L, final). Hydrolysis was calculated as indicated in the Methods section. The results show the hydrolysis (mean $\pm$ SEM, $n = 10$) of each indicated phospholipid expressed in nmoles per milliliter per minute. ** $p < 0.01$ versus PA (Wilcoxon test).



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TABLE I. Effect of EDTA and calcium on acetylhydrolase and PLA₂ activities of NLF

	Phospholipid hydrolysis		
	PA (mol/ml/min)	PG (mol/ml/min)	PAF (pmol/ml/min)
Saline solution	<1	<1	<0.1
NLF	<1	<1	52.8 ± 2.1
+EDTA (5 mmol/L)	<1	<1	62.0 ± 2.4*
+Calcium (5 mmol/L)	58.0 ± 28.2	126.8 ± 69.6	65.0 ± 3.1*

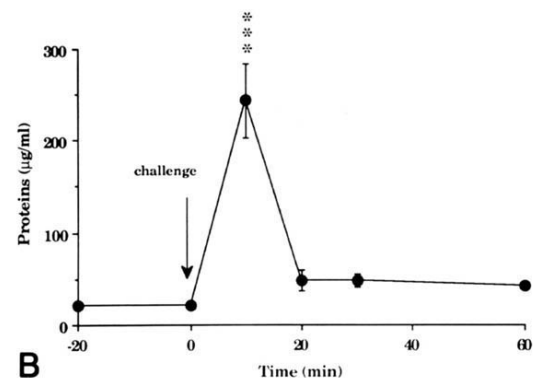
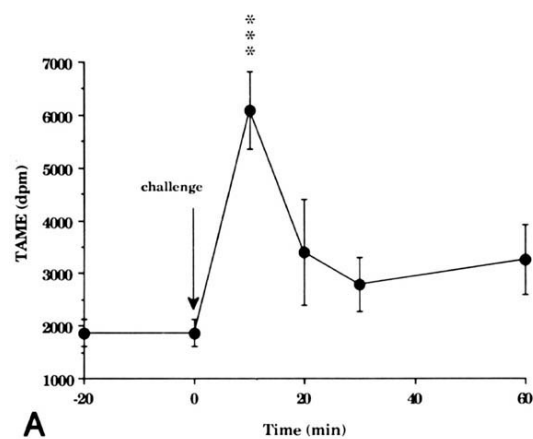
Aliquots of NLF collected 10 minutes after allergen challenge were thawed and immediately incubated with 5 mmol/L EDTA or 5 mmol/L CaCl₂. The hydrolysis of the phospholipid substrates was measured as indicated in the Methods section. Results are expressed as means ± SEM of six separate experiments.

*Not significant.

Failure of this enzyme to hydrolyze fluorescent PC is not due to the presence of an artificial acyl moiety at the sn-2 position of this substrate. Indeed, NLF failed to hydrolyze 1-palmitoyl-2-[¹⁴C]-arachidonoyl-PC (control assay showed that 0.4 µg of PLA₂ from *Crotalus durissus terrificus* cleaved more than 90% of added PC).

In parallel to acetylhydrolase and PLA₂ activities, allergen challenge provoked a secretion of TAME esterase and total proteins into the fluids recovered by nasal lavage. The TAME-esterase concentrations increased by 3.6 ± 0.6 times over the basal value and the total protein content increased more extensively (10.1 ± 2.1 times). The NLF content of TAME-esterase and proteins returned progressively to near basal value within approximately 1 hour (■ Fig. 5).

FIG. 5. Time course of TAME esterase **(A)** and total protein **(B)** release into NLF after allergen challenge. The allergen aerosol was applied to the patients, and then NLF was collected at the time points indicated. The arrows indicate the moment when allergen was applied to the patients. ***p* < 0.01 versus NLF before the challenge.

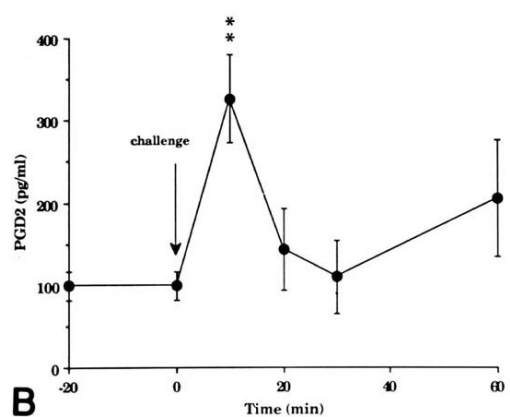
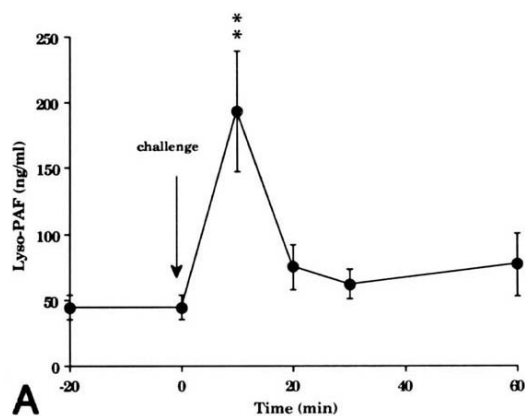


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Mediator release

Before the challenge with allergen, lyso-PAF was detected in the NLF from all of the patients. The allergen provocation induced an increase in this content, which peaked 10 minutes after the challenge to reach $423\% \pm 94\%$ over basal values. The identification of lyso-PAF in NLF was based on the following criteria: (1) the material comigrated with synthetic lyso-PAF; (2) it induced platelet aggregation only after chemical acetylation; (3) this aggregation was blocked by pretreating the platelets with the PAF antagonist, WEB 2086, but not with aspirin (data not shown). On the other hand, a parallel increase was observed in the concentration of PGD_2 recovered in NLF after allergen challenge. This increase reached its maximal level 10 minutes after the challenge ($370\% \pm 80\%$ over basal values) (■ Fig. 6, B). Lyso-PAF and PGD_2 concentrations of NLF returned to their basal values within 1 hour after the challenge. However, no significant amounts of PAF were detected in the NLF from 7 of 10 patients neither before nor after allergen challenge (data not shown). The concentrations of PAF found in the NLF of the other patients (3 of 10) were near the detection limit of our assay, varying from 0.08 to 0.29 ng/ml (0.15 ± 0.03 ng/ml $n = 3$). In the latter group of patients, the concentration of PAF did not change significantly after the challenge (data not shown).

FIG. 6. Time course of lyso-PAF (A) and PGD₂ (B) release into NLF after allergen challenge. The allergen aerosol was applied to the patients, and then NLF samples were collected at the time points indicated. The arrows indicate the moment when allergen was applied to the patients. The results show the concentrations (mean $\pm$ SEM) of lyso-PAF and PGD₂ in NLF. ** $p < 0.01$ versus NLF before the challenge.



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Discussion

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This study shows that allergen challenge of patients with allergic rhinitis leads to a rapid increase in NAR and nasal symptom scores associated with the release into the NLF of the proinflammatory mediator PGD₂. These findings are in agreement with those of previous studies, showing that nasal provocation with allergen induces the release of leukotrienes B₄, C₄, and D₄.^{6 8} Taken together, these results suggest that PLA₂ activation occurs in nasal tissues on allergen provocation. The release of TAME esterase into NLF confirms that activation of mast cells occurred after allergen provocation. The latter also led to a concomitant release into NLF of lyso-PAF, a precursor and/or metabolite of PAF, as previously demonstrated.^{8 9} The concentrations of PAF we detected in NLF were near or below the detection limit of our assay and are 500 to 1000 times lower than those of

lyso-PAF. The failure of allergen challenge to induce the release of PAF into NLF has already been reported in previous studies.^{8 9} This can be accounted for by its retention by nasal mucosal cells or by a rapid hydrolysis into inactive metabolite(s) by enzymes including acetylhydrolase. The latter is involved in the degradation of PAF in biologic fluids, including urine,^{21 22} blood,²³ and amniotic fluid.²¹ More recent studies have shown that low levels of plasma acetylhydrolase are associated with the severity of respiratory symptoms in children with asthma, potentially because of an accumulation of PAF.¹²

Indeed, we show here that allergen provocation led to the release into the nasal fluids of an enzymatic activity capable of hydrolyzing PAF with characteristics similar to those of acetylhydrolase. The hydrolysis of PAF by NLF is accompanied by a loss of its biologic activity, as assessed by platelet aggregation. Although no direct relationship between the absence of PAF and the increase of acetylhydrolase levels in NLF was established, we suggest a potential role for this enzyme in the inactivation of any PAF that may be released in lyso-PAF. This process could explain, at least in part, the relatively high levels of lyso-PAF found in NLF.

Our results also show that allergen challenge provoked the secretion of a calcium-dependent PLA₂ in nasal fluids. This enzyme preferentially hydrolyzes negatively charged phospholipids (PA and PG) versus PC. More interestingly, the hydrolysis of PA and PG by NLF was completely abolished by the addition of EDTA, which had no effect on the hydrolysis of PAF, indicating that the PLA₂ secreted in nasal fluids is not involved in the degradation of PAF. Alternatively, this enzyme could produce lyso-PAF through the hydrolysis of its precursor alkylacyl-PC.^{2 3} However, the fact that this PLA₂ preferentially hydrolyzes negatively charged phospholipids (which is not the case of alkylacyl-PC) is not in favor of this hypothesis and suggests that another PLA₂, probably located at the intracellular level, is involved in this reaction.

The characteristics of the PLA₂ we report here are similar to those of the type-II secretory PLA₂. The latter has been found in inflamed sites of rats, mice, and human beings.^{24 29} Other studies have demonstrated that the secretory PLA₂ triggers an acute inflammatory response when injected in rabbits.^{30 31} Although these secretory PLA₂s have proinflammatory roles, the mechanisms by which they contribute to inflammation and allergy remain to be established.

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- Received for publication May 18, 1993.
- Revised Sept. 27, 1993;
- Accepted for publication Dec. 30, 1993.
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