Platelet-activating factor (PAF) stimulates the production of PAF acetylhydrolase by the human hepatoma cell line, HepG2.

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Research Article

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Platelet-activating Factor (PAF) Stimulates the Production of PAF Acetylhydrolase by the Human Hepatoma Cell Line, HepG2

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Abstract

The human hepatoma cell line, HepG2, secreted an activity that degrades platelet-activating factor (PAF) by the hydrolysis of the *sn*-2 acetyl group. This activity was Ca^{++} independent, inhibited by diisopropylfluorophosphate but not by *p*-bromophenacyl bromide, and resistant to treatment with trypsin or pronase. Separation of HepG2-conditioned medium by gel filtration disclosed that the activity was associated with lipoproteins. An antiserum against PAF acetylhydrolase immunoprecipitated this activity. It was not recognized by an antibody against lecithin:cholesterol acyltransferase (LCAT), which also is secreted by HepG2 cells. Therefore the phospholipase A_2 activity of LCAT was excluded as a source of the observed activity. PAF added to the culture medium stimulated the secretion of the PAF-degrading activity by HepG2 cells, while lyso-PAF was inactive. Maximal stimulation was observed with 5 ng/ml PAF, which induced a fivefold increase. The presence of 5 ng/ml PAF enhanced the secretion of [^{35}S]methionine-labeled PAF acetylhydrolase and cycloheximide inhibited both the basal and PAF-stimulated secretion of the labeled enzyme.

We conclude that HepG2 cells produce PAF acetylhydrolase. The liver may be a major source of plasma PAF acetylhydrolase, and PAF may induce the production of its inactivating enzyme by the liver. (*J. Clin. Invest.* 1991. 87:476–481.) Key words: lipoproteins • lecithin:cholesterol acyltransferase (LCAT) • phospholipase A_2 • liver • metabolic labeling

Introduction

Platelet-activating factor (PAF)[1] is a bioactive phospholipid produced by various types of cells in response to an appropriate agonist (1, 2). PAF has been identified as 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (3) and is metabolized through the hydrolytic cleavage of the *sn*-2 acetyl moiety by a specific enzyme, PAF acetylhydrolase (4, 5). There are intra- and extracellular forms of this enzyme (6), and they may play an important role as a mechanism to regulate the levels of PAF. The activity of this enzyme in plasma has been reported to be higher in patients with dyslipoproteinemia (7), atherosclerotic diseases (8), ischemic stroke (9), and essential hypertension (10). It is reported to be lower in patients with severe bronchial asthma (11). It has also been suggested that a decreased plasma PAF acetylhydrolase activity in late pregnancy is related to the initiation of labor (12). However, the mechanism and significance of the alterations of plasma PAF acetylhydrolase activity have not been fully elucidated. In plasma this enzyme is associated with LDL and HDL (13, 14). This suggests that plasma PAF acetylhydrolase is produced and released by the liver, as a complex with lipoproteins. We examined the production of PAF acetylhydrolase by the human hepatoma cell line, HepG2 (15), which retains many aspects of normal functions of human hepatocytes including lipoprotein metabolism (16–22). Special reference was made to the differentiation of the PAF acetylhydrolase activity from the phospholipase A_2 activity of lecithin:cholesterol acyltransferase (LCAT), which is also produced by HepG2 cells (23). The effect of PAF on the production of PAF acetylhydrolase was also studied.

Methods

Chemicals. Culture medium, FCS, antibiotics, and other reagents for cell culture were obtained from Gibco Laboratories (Grand Island, NY). [2-Acetyl-^{3}H]PAF (370 GBq/mmol or 10 Ci/mmol), [4-^{14}C]cholesterol (2.22 MBq/mmol), and [^{35}S]methionine (46.07 TeraBq/mmol) were from New England Nuclear (Boston, MA). Unlabeled PAF, lyso-PAF, and other phospholipids were from Sigma Chemical Co. (St. Louis, MO), which also supplied apolipoprotein (apo) A-I, fatty acid-free HSA, diisopropylfluorophosphate (DFP), *p*-bromophenacyl bromide (BPB), and protein A-Sepharose CL 4B. Octadecylsilica gel cartridges (C_{18} Pre Sep) were purchased from Gasukuro Kogyo (Tokyo, Japan). Rabbit antisera against human apo A-I or apo B were obtained from Daiichi Pure Chemical (Tokyo, Japan). Rabbit antiserum against PAF acetylhydrolase (24) and a monoclonal antibody against LCAT (25) were generous gifts from Dr. Diana Stafforini (University of Utah) and Dr. Angelo Scanu (University of Chicago), respectively. Nonimmune rabbit IgG was from Zymed Laboratories (San Francisco, CA). A specific receptor antagonist of PAF, CV-3988 (26), was generously provided by the Central Research Division, Takeda Chemical Industries (Osaka, Japan).

Cell culture. HepG2 cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in 35-mm dishes (Falcon Labware, Oxnard, CA) using Eagle's MEM supplemented with 10% (vol/vol) FCS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin G, and 100 μg/ml streptomycin. Cells were incubated in a humidified atmosphere of 95% air and 5% CO_2. Viability of the cells was assessed by the trypan blue exclusion method (27).

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1. *Abbreviations used in this paper:* apo, apolipoprotein; BPB, *p*-bromophenacyl bromide; DFP, diisopropylfluorophosphate; GPC, glycero-3-phosphocholine; LCAT, lecithin:cholesterol acyltransferase; LDH, lactic dehydrogenase; PAF, platelet-activating factor; PC, phosphatidylcholine.

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After the cells had reached confluence, the monolayers were washed twice with HBSS, and fresh medium without FCS was added. After incubating for up to 36 h (usually 24 h), the medium was recovered and assayed for PAF acetylhydrolase activity. In the experiments to examine the effects of PAF or lyso-PAF, these phospholipids were dissolved in 20 mM PBS, pH 7.4, containing 2.5 mg/ml HSA, and 1/10 vol of either solution was added to the culture medium. The possible inhibition of the effect of PAF by PAF antagonist CV-3988 (26) was also tested. CV-3988 was dissolved in HBSS by warming to 50°C and the solution was added to the culture medium at the final concentration of 1 μM. HepG2 cells were incubated with CV-3988 for 1 h before the addition of PAF.

To obtain the sample for chromatographic separation of the enzyme, HepG2 cells were cultured in 75-cm^2 tissue culture flasks (Falcon Labware), and the supernatants from 10 flasks were combined and concentrated using Immersible CX-10 ultrafiltration units (Millipore Continental Water Systems, Bedford, MA).

PAF acetylhydrolase assay. PAF acetylhydrolase activity in the conditioned medium was assayed according to Stafforini and coworkers (28) with a slight modification. Each assay mixture contained 10–30 μl of HepG2-conditioned medium and 5 μl of substrate ([2-acetyl-^{3}H]PAF), and the final volume was adjusted to 50 μl with 0.1 M Hepes buffer, pH 7.2. The final concentration of the substrate was 80 μM. The substrate was prepared in Hepes buffer, by sonication, with a final specific activity of 5×10^4 dpm/nmol. Reactions were performed at 37°C for 30 min and stopped by adding 50 μl of 10 M acetic acid. This mixture was applied to an octadecylsilica gel cartridge, which was then washed three times with 1.0 ml of 0.1 M sodium acetate. The washes were collected into a scintillation vial and the radioactivity was determined. The effects of proteases, EDTA, and DFP on the PAF-degrading activity were tested as described (6, 28). The effect of BPB, an inhibitor of phospholipase A_2 (29), was also studied. BPB dissolved in ethanol was added to the conditioned medium and the mixture was incubated 30 min at room temperature. The final concentration of ethanol was 0.1%. The substrate specificity of the assay was examined by adding phospholipid molecules other than PAF. They were lyso-PAF, 1-hexadecyl-2-oleyl-*rac*-glycero-3-phosphocholine (1-hexadecyl-2-oleyl-GPC), egg yolk phosphatidylcholine (PC) and egg yolk phosphatidylethanolamine (PE). These phospholipids were solubilized in HBSS by sonication and added to the substrate solution.

Gel filtration chromatography. Chromatographic separation of HepG2-conditioned medium was performed using a fast protein liquid chromatography system (Pharmacia Fine Chemicals, Uppsala, Sweden) equipped with a gel permeation column TSK-GEL G5000PW + G3000SW × 2 (Toyo Soda, Japan) (30). Elution was performed with 20 mM Tris-HCl buffer, pH 7.4, at a flow rate of 0.2 ml/min. Fractions of 5 min were collected, and each fraction was assayed for PAF acetylhydrolase in a similar manner. Contents of apo A-I and apo B in each fraction were measured by single radial immunodiffusion using specific antisera.

LCAT assay. HepG2-conditioned medium was also assayed for LCAT activity according to Chen et al. (23) with a slight modification. A liposome with a phosphatidylcholine:cholesterol:apo A-I molar ratio of 250:12.5:0.6 was prepared by the cholate dialysis method (31), for use as the substrate. The specific activity of [^{3}H]cholesterol in this substrate was adjusted to 7.4 MBq/mmol. Each assay mixture contained 500 nmol phosphatidylcholine, 25 nmol cholesterol, 5% (wt/vol) HSA, 5 mmol 2-mercaptoethanol, 1.2 nmol apo A-I, and 200 μl of a sample. The total volume was adjusted to 1.0 ml with 10 mM Tris-HCl, pH 7.4, containing 140 mM NaCl and 1 mM EDTA. The reaction was performed for 2 h at 37°C and stopped by adding 3.25 ml of chloroform-methanol (2:1). Lipids were extracted and separated by thin layer chromatography as described by Verdery and Gatt (32). The area of silica gel corresponding to cholesterol ester was scraped into a vial and the radioactivity was measured by scintillation spectrometry.

Immunoprecipitation. To identify the PAF hydrolyzing activity as PAF acetylhydrolase, HepG2-conditioned medium was treated with either anti-PAF acetylhydrolase or anti-LCAT antibodies. Before use, these antibodies were treated with DFP to abolish the endogenous PAF acetylhydrolase activity and the remaining DFP was removed by dialysis against PBS. HepG2-conditioned medium was concentrated ~ 5-fold using an ultrafiltration unit. To this concentrate, an equal volume of the antibody solution, diluted 20-fold with PBS, was added and the mixture was incubated overnight at 4°C. To 200 μl of this mixture, 50 μl of 50% (vol/vol) suspension of protein A-Sepharose in PBS was added, and the mixture was further incubated for 1 h at 4°C. After brief centrifugation, the supernatant was assayed for PAF acetylhydrolase or LCAT.

Biosynthetic labeling of HepG2 proteins. HepG2 cells grown in a 35-mm dish were incubated with 1 MBq/ml [^{35}S]methionine in methionine-free MEM in the presence or absence of PAF (5 ng/ml). The effect of cycloheximide, an inhibitor of protein synthesis, was also studied. Cycloheximide was dissolved in HBSS and 10 μl of the solution was added to the culture medium to achieve a final concentration of 10 μg/ml. After 24 h, the conditioned medium was recovered. The total labeling of secreted proteins was determined using an aliquot (200 μl) of the conditioned medium. To 200 μl of the conditioned medium, 1 ml of albumin solution (5 mg/ml) was added and proteins were precipitated by adding 1 ml of 50% TCA. After centrifugation at 3,000 rpm for 10 min, the pellet was washed vigorously with distilled water, redissolved with 2 M NaOH and counted by scintillation spectrometry. The remainder of the conditioned medium was used for immunoprecipitation and electrophoresis.

Immunoprecipitation and electrophoresis of [^{35}S]methionine-labeled proteins. IgG was purified from the anti-PAF acetylhydrolase antiserum by protein A-Sepharose chromatography (33), dialyzed against PBS and finally diluted with PBS so that the volume would be 10 times of the original antiserum. To 800 μl of the conditioned medium, 10 μl of the IgG fraction was added and the mixture was incubated for 6 h at 4°C. Then 50 μl of 50% suspension of protein A-Sepharose was added and the mixture was further incubated for 1 h at 4°C. The mixture was centrifuged at 3,000 rpm for 10 min, and the pellet was washed 10 times with 10 ml of PBS. The final pellet was subjected to either counting with a scintillation counter or SDS-PAGE. For a control experiment, nonimmune rabbit IgG was substituted for the specific immune IgG.

SDS-PAGE was carried out on 10% slab gels (34). Immunoprecipitates were suspended in 50 μl of 25 mM Tris-glycine buffer (pH 8.3) containing 2% (wt/vol) SDS, 10 mM dithiothreitol, and 10% (vol/vol) glycerol, and heated for 3 min in a boiling water bath before being applied to the gel. Gels were run at 50 *V* constant voltage for ~ 1 h. Gels were fixed and stained as described (35). Gels were immersed for 30 min in 200 ml of DMSO twice and then in 100 ml of 20% (wt/wt) diphenyloxazole in DMSO for 3 h. After soaking in 200 ml of distilled water for 1 h, the gel was dried and exposed to a Kodak X-Omat film at −80°C for 7 d.

Lactic dehydrogenase as a cytotoxicity assay. Possible cytotoxic effects of PAF or cycloheximide were checked by monitoring the release of lactic dehydrogenase (LDH) into the conditioned medium (36). LDH activity was determined using a commercially available reagent kit (Wako Pure Chemicals, Osaka, Japan).

Quantification of cellular protein. Monolayers of HepG2 cells were solubilized by incubating them with sodium hydroxide solution. To a 35-mm dish, 2 ml of 0.2 M sodium hydroxide was added and the dish was incubated at room temperature for 1 h. Then the solution was recovered and the dish was washed three times with 1 ml of the sodium hydroxide solution. All of these solutions were combined and further incubated at room temperature for 1 h. An aliquot (50–200 μl) of this solution was used for protein quantification according to Lowry et al. (37).

Results

The release of [^{3}H]acetate from [2-acetyl-^{3}H]PAF by incubation with HepG2-conditioned medium is shown in Fig. 1. This

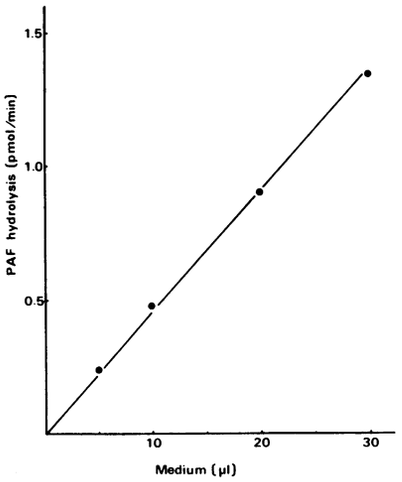

Figure 1. PAF hydrolyzing activity in HepG2-conditioned medium. The culture medium conditioned by HepG2 cells for 24 h was assayed for [2-*acetyl*-^{3}H]PAF degrading activity as described in Methods. This activity was found to be linear with the amount of sample ranging from 5 to 30 μl.

reaction was dependent on the period of incubation and on the amount of medium up to a protein content in the assay of 20 μg.

The effect of DFP, EDTA, BPB, proteases, and various phospholipids on the activity are summarized in Table I. DFP inhibited the PAF acetylhydrolase activity in a concentration-dependent manner and the inhibition at 1 mM was almost complete. EDTA and BPB had no effect on the PAF acetylhydrolase activity in HepG2-conditioned medium. Preincubation of the medium with either trypsin or pronase also did not affect the activity. The possible effect of protease inhibitors in the medium was excluded by a control experiment in which more than 90% of added protease activity was detected, by a caseinolytic assay, in the samples after the incubation (data not shown). Lyso-PAF or other phospholipids also did not affect the assay.

Table I. Effects of DFP, EDTA, BPB, Proteases, and Various Forms of Phospholipids on the PAF Acetylhydrolase Activity in HepG2-conditioned Medium

Treatment		PAF acetylhydrolase (percent of control)
DFP	0.1 mM	32, 54
	1.0 mM	8, 4
EDTA	10 mM	100, 113
BPB	10 μM	115, 113
Trypsin*	1 mg/ml	102, 109
Pronase*	1 mg/ml	111, 98
Egg yolk PC‡	0.1 mM	89, 94
	0.5 mM	95, 100
Egg yolk PE‡	0.1 mM	97, 103
	0.5 mM	96, 98
1-Hexadecyl-2-oleyl-GPC‡	0.1 mM	102, 105
	0.5 mM	95, 100

HepG2 cells were incubated in serum-free medium for 24 h and the conditioned medium was assayed for PAF acetylhydrolase. The values of two separate experiments, each with duplicate determinations, are shown. * HepG2-conditioned medium was incubated with the proteases for 30 min at 37°C before addition of the substrate for assay of PAF acetylhydrolase activity. ‡ These phospholipids were dissolved in PBS by sonication and added to the substrate solution.

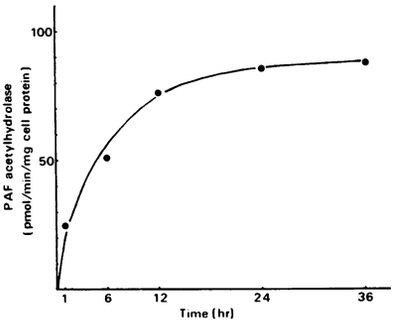

Figure 2. Time-dependent secretion of PAF acetylhydrolase by HepG2 cells. HepG2 cells were cultured for up to 36 h in serum-free medium. PAF acetylhydrolase activity in the conditioned medium increased gradually until 24 h and plateaued thereafter.

Production of PAF acetylhydrolase by HepG2 cells as a function of time in culture is shown in Fig. 2. PAF acetylhydrolase activity in the medium plateaued at 24 h of incubation, after which time the activity was unchanged for up to 36 h. After 36 h of incubation in the medium without FCS, the viability of the cells tested by a dye exclusion assay was 92%, which was slightly lower than that of the cells at 24 h: 97% (n = 2). Thus the decreased secretion of the activity after 36 h of incubation may have been due to the poor ability of the serum-free medium to support normal cellular functions. Therefore, in the following experiments HepG2 cells were incubated in the serum-free medium for 24 h.

Separation of HepG2-conditioned medium by gel permeation high performance liquid chromatography is shown in Fig. 3. PAF acetylhydrolase activity was recovered in the fractions that had an elution volume that corresponded to that of plasma lipoproteins. The elution of the activity coincided with the fractions that contained either apo A-I or apo B.

The results of immunoprecipitation experiments are summarized in Table II. When the conditioned medium was precipitated with the anti-PAF acetylhydrolase antiserum and protein A-Sepharose, the supernatant did not hydrolyze PAF, whereas most of the LCAT activity remained in solution. The immunoprecipitation using the anti-LCAT antibody resulted in the opposite pattern: LCAT was precipitated by the antibody while PAF acetylhydrolase activity was not.

The effect of PAF on the secretion of PAF acetylhydrolase by HepG2 cells is shown in Fig. 4. Under basal conditions, HepG2 cells secreted PAF acetylhydrolase at a rate such that at the end of 24 h there was a total activity of 12±14 pmol/min per mg cell protein. PAF at concentrations of 0.1–10 ng/ml (0.18–18 nM) stimulated the secretion of the enzyme and the maximal effect was observed with 5 ng/ml (9 nM) PAF, which induced an approximately fivefold increase. PAF was inhibitory at 50 ng/ml. At this concentration, PAF was found to be toxic to the cells since the dye exclusion test disclosed only 82% cell viability after 24 h of incubation (n = 2). Lyso-PAF or egg yolk PC, added to the culture medium at a concentration of either 5 or 50 ng/ml did not affect the secretion of PAF acetylhydrolase by HepG2 cells (data not shown). The stimulatory effect of 5 ng/ml (9 nM) PAF was abolished by pretreating HepG2 cells with 1 μM CV-3988 for 1 h (Table III).

The results of biosynthetic labeling are shown in Table IV. Under basal conditions, HepG2 secreted [^{35}S]methionine-labeled PAF acetylhydrolase of 3.9±1.8 fmol/mg cell protein in 24 h (n = 7), which corresponded to 0.22% of the total secreted proteins labeled. Labeling of PAF acetylhydrolase was increased significantly ($P < 0.01$) by 5 ng/ml PAF: 9.6±4.2 fmol/mg cell protein, and this corresponded to only 0.30% of the

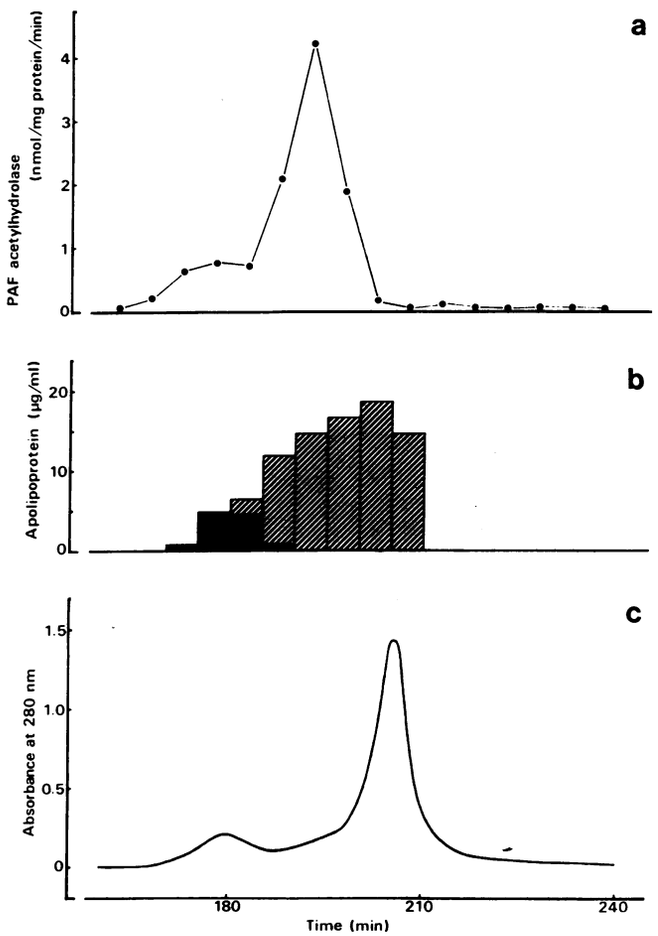

Figure 3. Gel filtration chromatography of the HepG2-conditioned medium. The conditioned medium recovered from twenty 75-cm^2 flasks was concentrated, and then fractionated by a fast protein liquid chromatography system equipped with a gel filtration column (TSK GEL G5000PW + G3000SW × 2). Elution was performed with 10 mM phosphate buffer (pH 7.2) at a flow rate of 0.2 ml/min. Fractions of 10 ml were collected and assayed for PAF acetylhydrolase activity. (*a*) PAF acetylhydrolase activity. (*b*) Contents of apo A-I (*hatched columns*) and apo B (*black columns*). (*c*) Elution profile of protein as monitored by absorption at 280 nm.

total labeling because PAF enhanced the labeling of total secreted proteins as well. Cycloheximide inhibited the incorporation of [^{35}S]methionine in secreted proteins and abolished the stimulatory effect of PAF. These effects of PAF and cycloheximide were also confirmed by SDS-PAGE as shown in Fig. 5. Although a few minor bands were seen on the fluorogram of the gel, there was an obvious increase in the density of the major band around 45,000 D, which is consistent with plasma PAF acetylhydrolase (24). The effects of PAF and cycloheximide were not due to cytoxicity of these compounds since the extracellular release of LDH, an indicator of cytolysis (36), was not increased in the presence of PAF and/or cycloheximide (Table IV).

Discussion

In this study, we demonstrated the production of PAF acetylhydrolase by HepG2 cells. PAF acetylhydrolase is a degrading

Table II. Immunological Identification of PAF Acetylhydrolase in HepG2-conditioned Medium

Antibody	Activity (percent of control)	
	PAF acetylhydrolase	LCAT
Anti-PAF acetylhydrolase	3±2.7	89±20.4
Anti-LCAT	108±11.3	11±2.9

HepG2-conditioned medium was incubated with either anti-PAF acetylhydrolase or anti-LCAT overnight, a suspension of protein A-Sepharose was added, and the supernatant was assayed for PAF acetylhydrolase or LCAT. In the control experiment the conditioned medium was incubated with nonimmune rabbit serum or PBS. Data are means (±SD) of three experiments each with duplicate determinations. The average values of PAF acetylhydrolase and LCAT activity in control experiments were 9±3.2 pmol/min per mg cell protein and 0.9±0.28 nmol/h per mg cell protein, respectively.

enzyme specific for PAF since it is inactive against similar compounds with a side chain longer than four carbons at the *sn*-2 position of the molecule (5). This enzyme does not require Ca^{++}, and is inactivated irreversibly by DFP. Both intra- and extracellular forms of the enzyme are known, and the former, but not the latter, is inactivated by hydrolysis with trypsin and other proteases (6). Inhibition by DFP, Ca^{++} independence, and resistance to pretreatment with trypsin or pronase support the identification of the enzyme secreted by HepG2 cells as the extracellular form of PAF acetylhydrolase. Although PAF is a substrate for the more common form of phospholipase A_2 (38), this enzyme does not account for the activity in HepG2-conditioned medium since BPB and exclusion of Ca^{++} from the reaction mixture did not affect the activity. The identification of this enzyme as PAF acetylhydrolase is also supported by the results of experiments examining the substrate specificity. The presence of an excess of phospholipids other than PAF in the reaction did not interfere with the hydrolysis of PAF.

Separation of HepG2-conditioned medium by gel filtration disclosed that PAF acetylhydrolase had an apparent molecular weight similar to plasma lipoproteins and that the activity was

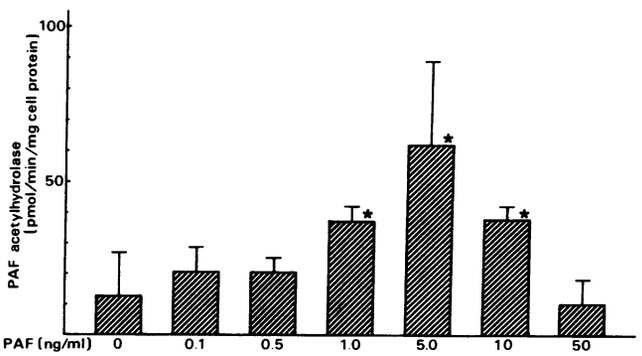

Figure 4. The effect of PAF on the secretion of PAF acetylhydrolase by HepG2 cells. PAF was dissolved in PBS containing 2.5 mg/ml fatty acid-free HSA and added to the serum-free culture medium. After 24 h the medium was recovered and assayed for PAF acetylhydrolase. Average values of five experiments are shown. The vertical bars indicate standard deviation. *Significantly higher than the control value ($P < 0.01$, based on Student's *t* test).

Table III. Effect of CV-3988 on the PAF-induced Stimulation of the Production of PAF Acetylhydrolase by HepG2 Cells

CV-3988	PAF	PAF acetylhydrolase (percent of control)
μM	*nM*	
1	0	112±16.7
0	9	270±47.6
1	9	93±22.0

HepG2 cells were incubated with serum-free medium containing 1 μM CV-3988 for 1 h before the addition of PAF. After 24 h the conditioned media were collected and assayed for PAF acetylhydrolase activity. Data are means (±SD) of three experiments each with duplicate determinations. The mean±SD of PAF acetylhydrolase activity in control experiments was 25±9.7 pmol/min per mg cell protein.

coeluted with apolipoproteins. These results imply that HepG2 cells secrete PAF acetylhydrolase as a complex with lipoproteins.

HepG2 cells have been shown to produce LCAT (23) and the phospholipase A_2 activity of this enzyme could have accounted for the PAF hydrolysis by HepG2-conditioned medium. In the gel permeation chromatography LCAT, in the conditioned medium, has been separated with a molecular size (23) similar to the PAF hydrolyzing activity found in this study. However, this possibility was excluded since the PAF hydrolyzing activity was almost completely precipitated with an antibody against PAF acetylhydrolase while an antibody against LCAT did not exert any effect.

Plasma PAF acetylhydrolase activity has been shown to be higher in patients with dyslipoproteinemia (7) and atherosclerotic peripheral vascular disease (8), and lower in asthma (11). Also, this enzyme activity in plasma has been shown to correlate with the levels of LDL (9, 39). In our previous studies, plasma PAF acetylhydrolase activity was higher in patients with ischemic stroke (9) or essential hypertension (10) without any significant changes in plasma lipoproteins. We suggested that the increased enzyme activity in plasma of these patients

Table IV. Effects of PAF and Cycloheximide on the Incorporation of [^{35}S]Methionine into PAF Acetylhydrolase Secreted by HepG2 Cells

PAF	Cycloheximide	Specific labeling	LDH release
nM	*μg/ml*	*fmol/mg cell protein*	*U/mg cell protein*
0	0	3.9±1.8 (0.22)*	147±16
0	10	0.5±0.2 (0.40)	125±13
9	0	9.6±4.2 (0.30)*	101±18
9	10	1.2±0.4 (0.24)	132±30

HepG2 cells were incubated for 24 h with 1 MBq/ml [^{35}S]methionine in serum- and methionine-free MEM. The conditioned medium was collected, and PAF acetylhydrolase was separated by immunoprecipitation and counted. Each value represents mean±SD of seven experiments each with duplicate determinations. The numbers in parentheses are percent of total labeling of secreted proteins. * $P < 0.01$.

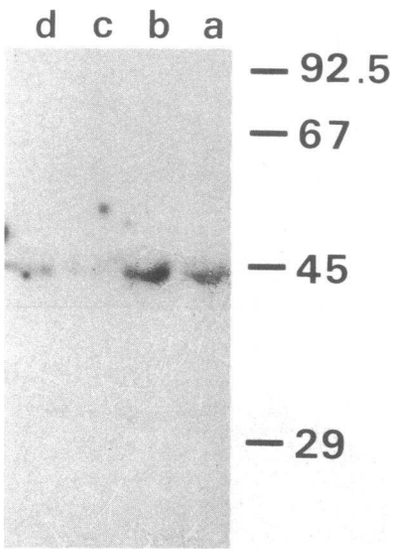

Figure 5. Incorporation of [^{35}S]methionine into PAF acetylhydrolase secreted by HepG2 cells. HepG2 cells were incubated with [^{35}S]-methionine and PAF acetylhydrolase were separated by immunoprecipitation using anti-PAF acetylhydrolase antibody and protein A-Sepharose. The immunoprecipitates were separated by SDS-PAGE. The gel was enhanced with diphenyloxazole, dried, and exposed to a film for 7 d at −80°C. Lane *a*, control; lane *b*, Hep G2 cells labeled in the presence of 9 nM PAF; lane *c* and *d*, 10 μg/ml cycloheximide was added in the presence (*c*) or absence (*d*) of 9 nM PAF.

was an adaptation to an increased PAF production (9, 10). Although such a view has to be tested by further studies, our finding of the enhanced PAF acetylhydrolase secretion in response to PAF supports our previous hypothesis. This effect of PAF is specific since it was abolished by CV-3988 and lyso-PAF or egg yolk PC did not stimulate the secretion.

The stimulatory effect of PAF on the production of PAF acetylhydrolase was confirmed by metabolic labeling with [^{35}S]methionine. PAF enhanced the secretion of [^{35}S]methionine-labeled PAF acetylhydrolase, and inhibition of protein synthesis with cycloheximide blocked both the basal and PAF-stimulated production of the enzyme. Since the presence of these compounds during conditioning did not increase the release of LDH, the possible liberation of intracellular PAF acetylhydrolase due to cytolysis was excluded.

In summary, HepG2 cells produce PAF acetylhydrolase and secrete it as a complex with lipoproteins, and PAF stimulates this secretion. The liver may be one of the important sources of plasma PAF acetylhydrolase.

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