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Biosynthesis of platelet-activating factor by cultured rat Kupffer cells stimulated with calcium ionophore A23187

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Cultured rat Kupffer cells synthesize and release platelet-activating factor (PAF) when stimulated with calcium ionophore A23187. The production of PAF is concentration- and time-dependent and, based upon [^{3}H]serotonin release assays, approx. 1.0 pmol of PAF is formed per 8×10^6 cells during 10 min of ionophore stimulation. It is suggested that Kupffer cells are important cellular components which produce and release PAF in order to facilitate communication between hepatic sinusoidal and parenchymal cells. Further, it is suggested that such mediator production in response to reticulo-endothelial cell stimulation causes the hepatic glycogenolytic response reported previously in the isolated perfused rat liver.

INTRODUCTION

PAF (platelet activating factor; AGEPC, identified primarily as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) [1,2], is a unique phospholipid described initially as a potent mediator of allergic reactions [3,4]. Since its initial identification, PAF has been shown to have diverse biological activities including induction of platelet aggregation and secretion [4,5], chemotaxis and degranulation of polymorphonuclear leukocytes [6], negative inotropic effects in guinea pig heart [7], hypotension [8] and vasopermeability [9]. PAF is produced under a variety of stimuli in various types of cells and tissues, such as human and rabbit platelets [4,10], neutrophils [11–13], basophils [14–16], peritoneal and alveolar macrophages [17–20], isolated rat kidney cells [21], cultured human endothelial cells [22–26], perfused guinea pig heart [7], perfused rat liver [27,28] and rabbit lung [29].

When PAF, at concentrations ranging from 1×10^{-9} – 1×10^{-11} M, is infused into perfused livers of well-fed rats, stimulation of glycogenolysis and increased portal vein pressure are observed [30–32]. These effects are transient and both agonist-concentration- and perfusate-calcium-dependent. However, lack of PAF-induced glycogenolysis in isolated hepatocytes and liver slices has been observed although significant glycogenolytic responses could be elicited by both vasopressin and glucagon in the same preparations [33]. Interestingly, several substances, e.g. heat-aggregated IgG [27] or zymosan [28], which are thought to stimulate reticulo-endothelial cells in the liver, cause vasoconstriction and glycogenolysis when infused in a manner similar to that seen with PAF. Moreover, these particulate substances stimulate formation of PAF in the perfused rat liver [27,28]. In addition, infusion of [^{3}H]PAF into the perfused rat liver, followed by tissue sectioning and mounting on slides coated with a photographic emulsion, showed that most of the [^{3}H]PAF was localized in the portal sinusoids instead of in parenchymal cells [34]. These observations suggest that the reticulo-endothelial cells, e.g. endothelial cells and/or Kupffer cells, are the site of initial binding and formation of this potent phospholipid mediator in rat liver and could play an important role in the hepatic metabolism and activities of PAF.

In the present study, the biosynthesis of PAF by cultured rat Kupffer cells stimulated with calcium ionophore A23187 was investigated. It was demonstrated that during stimulation with calcium ionophore A23187, cultured rat Kupffer cells synthesize and release PAF which is shown to have identical biological and chromatographic characteristics to authentic synthetic PAF.

MATERIALS AND METHODS

Materials

1-*O*-[1′,2′-^{3}H]alkyl-2-acetyl-*sn*-glycero-3-phosphocholine ([^{3}H]PAF; 59.5 Ci/mmol) and the sodium salt of [^{3}H]acetate (3.4 Ci/mmol) were purchased from New England Nuclear, Boston, MA, U.S.A. 1-*O*-[^{3}H]-octadecyl-2-lyso-*sn*-glycero-3-phosphocholine ([^{3}H]-lyso-PAF; 80 Ci/mmol) was purchased from Amersham, Arlington Height, IL, U.S.A. The purity of [^{3}H]PAF and [^{3}H]lyso-PAF was greater than 93%, as determined by t.l.c. The free acid form of the calcium ionophore A23187 was purchased from CalBiochem, San Diego, CA, U.S.A., and dissolved in dimethyl sulphoxide (DMSO) (Fisher Scientific, Pittsburgh, PA, U.S.A.). The lithium salt of acetyl coenzyme A (acetyl-CoA) was the product of Pharmacia, Piscataway, NJ, U.S.A. Polar lipid standards were from Avanti Polar Lipids Inc., Birmingham, AL, U.S.A., and unlabelled PAF from BACHEM BioScience, Inc., Bubendorf, Switzerland. Fatty-acid-free bovine serum albumin was from Boehringer Mannheim Biochemicals, Indianapolis, IN, U.S.A. Metrizamide (2,3-acetamido-5-*N*-methyl-acetamido-2,4,6-tri-iodobenzamido)-2-deoxy-D-glucose)

Abbreviations used: PAF, platelet-activating factor; AGEPC, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; lyso-PAF, 1-*O*-alkyl-2-lyso-GEPC; SPH, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TNS, toluidinyl-2-naphthalene sulphonate; DMSO, dimethyl sulphoxide; alkylacyl-GPC, 1-O-alkyl-2-acyl-*sn*-glycero-3-phosphocholine.

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was purchased from NYCOMED Co., Oslo, Norway. Collagenase IX and protease E were from Sigma, St. Louis, MO, U.S.A.

Isolation and primary culture of rat Kupffer cells

Purified rat Kupffer cell suspensions were prepared using a modification of a previously-described method [35,36]. Briefly, the liver of a male Sprague–Dawley rat weighing 200–300 g was perfused *in situ* through the portal vein with 200–300 ml of Ca^{2+}/Mg^{2+}-free Krebs–Henseleit buffer [37] containing 0.1% bovine serum albumin (w/v), 0.2% glucose and antibiotics (penicillin and streptomycin) at 37 °C for nearly 10 min at a flow rate of 30 ml/min in a non-recirculating system. Then, 0.03% (w/v) collagenase and 0.02% (w/v) protease E together in 100 ml of the same buffer were perfused into the liver and recirculated for about 3–5 min at 37 °C. The liver was excised and the capsule removed. The liver was gently shaken in cold Krebs–Henseleit bicarbonate buffer and this suspension was filtered through nylon gauze and the filtrate centrifuged at 50 *g* for 45 s at 4 °C. The supernatant was collected and the above step was repeated twice. The non-parenchymal cells freed from erythrocytes and cell debris were obtained by centrifuging the supernatant at 4 °C, 220 *g* for 6 min, resuspending the cell pellet in 24 ml of Gey's balance buffer [45] without NaCl but containing 30% (w/v) metrizamide, and centrifuging at 4 °C, 1400 *g* for 20 min. Subsequently 15 ml of non-parenchymal cell suspension was transferred into a sample vial of the JE-6B elutriation system (Beckman Instruments, Irvine, CA, U.S.A.) and then washed into and separated in the sample chamber of the rotor with the Krebs–Henseleit bicarbonate buffer at flow rates of 21.5 ml/min and 42.5 ml/min at 15 °C. The rotor speed was 2500 rev./min. Purified Kupffer cells, which were collected in 300 ml of Krebs–Henseleit bicarbonate buffer at a flow rate of 42.5 ml/min, were washed and resuspended in RPMI 1640 medium (GIBCO, Grand Island, NY, U.S.A.) containing 20% fetal bovine serum (Sigma). Either 8×10^6 cells in 4 ml of medium were plated in a 60 mm diam. Petri dish or 2×10^6 cells in 2 ml of medium were plated in a 35 mm diam. Petri dish. All dishes were incubated in a CO_2 incubator containing 95% air and 5% CO_2. The viability and the characteristics of the Kupffer cells were determined by testing for their ability to exclude Trypan Blue and their peroxidase activity, respectively. The purity of the Kupffer cell suspensions obtained in these experiments was 90–95%. The viability was 93 ± 2.8% ($n = 6$). All Kupffer cell preparations were used for experiments following three days in culture.

Lipid extraction and characterization of platelet-activating activity in ionophore A23187-stimulated Kupffer cells

The RPMI 1640 medium in 2–3 Petri dishes (60 mm diam.) was carefully removed from the cells and the cells were rinsed three times with 4 ml of fresh Hanks' buffer. Finally, 3 ml of Hanks' buffer, containing 0.25% (w/v) fatty acid-free bovine serum albumin was added to each plate and the cells were incubated for 10 min at 37 °C. The cultured cells were then stimulated with ionophore A23187 (5 μM) for the desired time period. The final concentration of DMSO in Hanks' buffer was 0.1% in all experimental and control incubations. The various incubations were terminated by adding an appropriate amount of methanol (7.5 ml) to each plate and the lipids were extracted essentially according to the procedure of Bligh & Dyer [38]. The extracted lipids were subjected to preparative t.l.c. on Silica Gel G plates (Uniplate, Analtech, Newark, NJ, U.S.A.). Chromatography was performed using a solvent system of chloroform/methanol/water (65:35:7, by vol.) as described previously [1,39,40]. A standard polar lipid mixture which contained authentic phosphatidylcholine (PC), sphingomyelin (SPH), and lyso-phosphatidylcholine (lyso-PC) was applied to the centre and left or right lanes of the same plate. These phospholipid 'markers' were detected by toluidinyl-2-naphthalene sulphonate spray [41]. PAF was located on the t.l.c. plate between the area corresponding to SPH (R_F value = 0.28) and lyso-PC ($R_F = 0.14$). The area corresponding to synthetic PAF ($R_F = 0.21$) was removed by scraping and extracted. The PAF activity of the lipid extracted in this fashion was determined by testing both aggregation of and also [^{3}H]-serotonin release from washed rabbit platelets which had been previously loaded with [^{3}H]serotonin [2]. Subsequent base treatment of the lipid extract having PAF activity led to a complete loss of biological activity which could be restored upon acetylation with acetic anhydride in the presence of catalytic amounts of perchloric acid [2].

Assay of [^{3}H]acetate incorporation into PAF

Experimental assessment of [^{3}H]acetate incorporation into PAF was accomplished as described by Mueller *et al.* [12] and Prescott *et al.* [23]. Briefly, the culture medium was removed from the 60 mm diam. culture dishes and replaced with 3 ml of Hanks' buffer containing 75 μCi of [^{3}H]acetate and incubated for 10 min at 37 °C. Calcium ionophore A23187 (5 μM final concentration) was added and incubated with Kupffer cells for another 10 min. The incubation was stopped by quickly removing the Hanks' buffer medium and immediately adding 7.5 ml methanol to the plates. Lipids in the cell and medium fractions were extracted separately as described above. The extracted samples, which were mixed with standard polar lipids, were separated by t.l.c. on Silica gel H (Uniplate, Analtech, Newark, NJ, U.S.A.) in the same solvent system as mentioned above. The pattern of radioactive labelling was determined by a combination of iodine vapour and individual scraping of spots, and by measuring the radioactivity by liquid-scintillation spectrometry. In some experiments, the areas co-migrating with authentic PAF were scraped, extracted and analysed by h.p.l.c. as indicated below.

H.p.l.c. analysis of the [^{3}H]acetate-labelled polar lipids extracted from Kupffer cells

The h.p.l.c. analysis of the total lipid extract was performed as described by Blank & Snyder [42]. It was demonstrated that all polar lipids tested in the experiment were well separated (Fig. 1). A more detailed description is given in the legend to Fig. 1.

Assay of [^{3}H]lyso-PAF incorporation into PAF by stimulated rat Kupffer cells

This experiment was performed using a modification of a previously described method [23]. Kupffer cells cultured in 35 mm diam. Petri dishes were used in the experiment. The maintaining culture medium was discarded and the plates rinsed three times with Hanks'

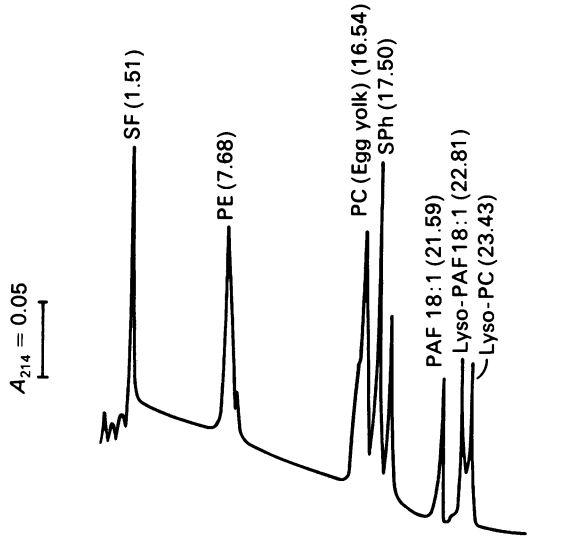

Fig. 1. H.p.l.c. separation of polar lipids mixture

The polar lipid standards indicated in the figure were injected onto a 4.6 mm × 250 mm Ultratechsphere 5SIL column and eluted with a mobile phase (2 ml/min) that initially consisted of 96% (v/v) solvent A (hexane/isopropanol, 1:1, v/v) and 4% (v/v) water. Up to 15 min after injection the amount of water was increased to 8% in a linear gradient. SF, solvent front.

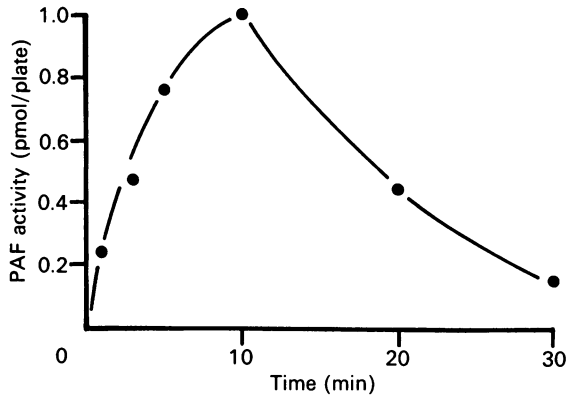

Fig. 2. Time-course of the production of PAF by rat Kupffer cells stimulated with 5 μM ionophore A23187 at 37 °C

The amount of PAF produced by Kupffer cells was determined by [^{3}H]serotonin secretion assay (see Materials and methods for details). Each data point is the mean value of PAF measurements from two cell preparations.

buffer. Serum-free culture medium (1 ml) containing 1 μCi of [^{3}H]lyso-PAF and 100 μM of acetyl-CoA were added to each dish and incubated for 40 min at 37 °C. The radioactive medium was removed and replaced with serum-free culture medium containing either ionophore A23187 dissolved in DMSO or DMSO alone. After various periods of incubation the reaction was terminated and the lipids were extracted from either cells or medium and analysed as described above.

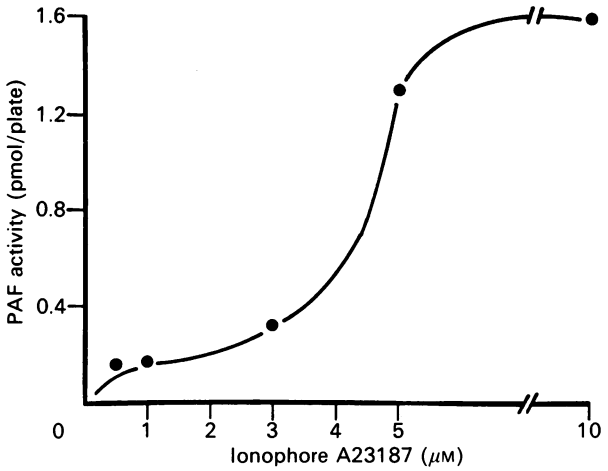

Fig. 3. Dose–response for the increases in PAF production by rat Kupffer cells in response to calcium ionophore A23187

8×10^6 cells were incubated with various concentrations of ionophore A23187 for 10 min and the reaction was terminated as described under Materials and methods. Each data point is the mean value of PAF measurements from two cell preparations.

RESULTS

General observations

Under conditions where Kupffer cells were stimulated with calcium ionophore A23187 (5 μM), it was shown clearly that PAF was produced by these cells in a time-dependent manner (Fig. 2). In these experiments, the peak production of PAF, i.e. approx. 1.0 pmol per 8×10^6 cells, occurred around 10 min after the addition of ionophore and rapidly declined thereafter. If 10 μM ionophore was used, the PAF level rose to 1.66 pmol per 8×10^6 cells, which was the maximum level of PAF measured (Fig. 3). When only DMSO was added (control) there was no detectable production of PAF.

Further identification of PAF in extracted lipid fractions, isolated using t.l.c., was obtained by subjecting the PAF fractions to base-catalysed methanolysis. This treatment led to complete loss of biological activity (Fig. 4, d), which could be restored by treatment of the presumed lyso-GEPC fragment with acetic acid anhydride and perchloric acid (Fig. 4, c).

Facets of the biosynthesis of PAF by Kupffer cells

Using [^{3}H]acetate as a labelled precursor of PAF, the incorporation of label into the total lipids of the Kupffer cells was examined. Fig. 5 illustrates the pattern of incorporation of [^{3}H]acetate, primarily into the phosphatidylethanolamine and phosphatidylcholine components of the A23187-treated as well as the DMSO-treated (control) set of cells. Interestingly, approx. 4.5% and 2.9% of the total radioactivity found in the lipids co-migrated with the PAF fraction on the t.l.c. plates in the A23187- and DMSO-treated cells, respectively. However, the biological activity of these radiolabelled lipids was not further assayed. When the cellular and medium fractions were analysed separately, we found that at least 70% of the [^{3}H]acetate-labelled PAF was released into the medium containing 0.25% bovine serum albumin (Table 1). After an initial purification on

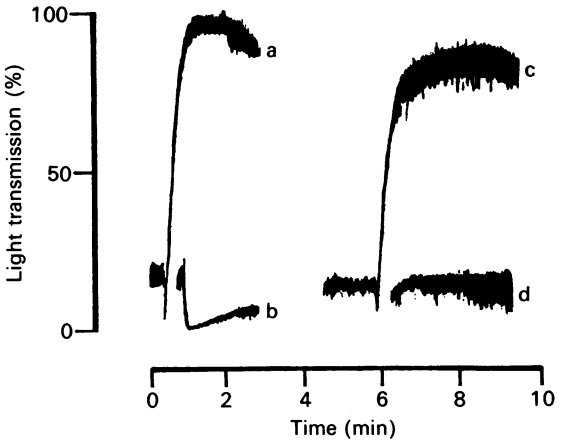

Fig. 4. Platelet aggregation induced by PAF from rat Kupffer cells

(a) Cells were stimulated with 5 μM-calcium ionophore A23187 for 10 min at 37 °C; (b) Kupffer cells were incubated with DMSO alone and showed no platelet aggregation activity. (d) Base treatment led to complete loss of biological activity of sample (a); and (c) platelet aggregation activity was restored upon acetylation.

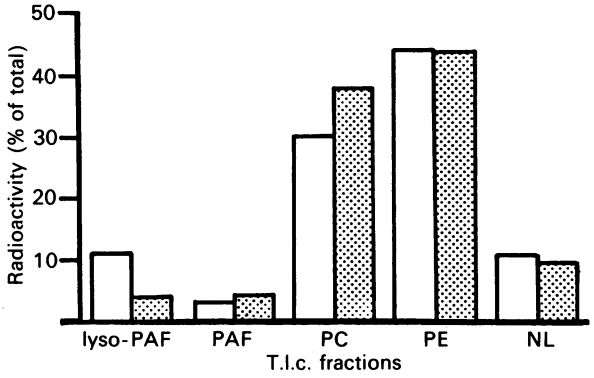

Fig. 5. Assay of [^{3}H]acetate incorporation into PAF

Rat liver Kupffer cells (8×10^6) were incubated for 10 min in 3 ml of Hanks' buffer containing 75 μCi of [^{3}H]acetate; ionophore A23187 (final concentration 5 μM) was added to several of the plates (▨) and DMSO only to controls (□). Lipids were extracted and analysed by t.l.c. The results shown here represent the mean value of duplicate plates from a single cell preparation. PC, phosphatidylcholine; PE, phosphatidylethanolamine; NL, neutral lipids.

silica gel H plates, the lipid extracts from the medium of ionophore A23187- and DMSO-treated rat Kupffer cells were subjected to h.p.l.c. analysis. The data in Fig. 6 show that the PAF fraction from ionophore A23187-stimulated Kupffer cells had the same retention time as either authentic [^{3}H]PAF or unlabelled PAF ($C_{18:1}$) (Fig. 1), whereas the same fraction from control t.l.c. plates did not have this chromatographic feature.

Effect of ionophore A23187 on the incorporation of [^{3}H]lyso-PAF into PAF and the metabolism of [^{3}H]lyso-PAF by rat Kupffer cells in the presence of acetyl-CoA

Cultured rat Kupffer cells which were challenged with

Table 1. Production of [^{3}H]acetate-labelled PAF by ionophore A23187-stimulated rat Kupffer cells

[^{3}H]Acetate incorporation was studied as described in Materials and methods, and the legend to Fig. 5. After 10 min of stimulation by 5 μM ionophore A23187 or DMSO alone the Kupffer cells were separated from Hanks' buffer and assayed separately.

Conditions	Radioactivity in PAF fraction on t.l.c. (d.p.m)
Ionophore A23187	
Released	19244
Cell-associated	8734
Control	
Released	3304
Cell-associated	5507

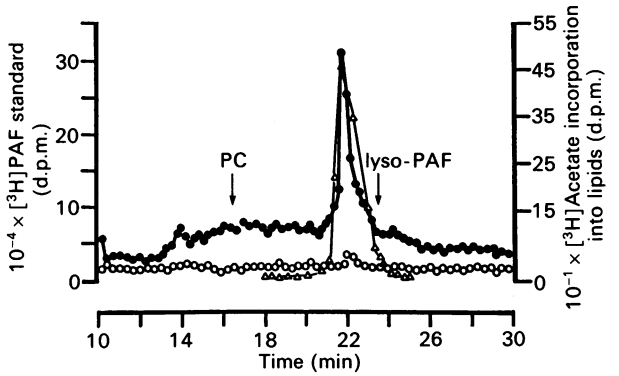

Fig. 6. H.p.l.c. analysis of [^{3}H]acetate-labelled lipids from Kupffer cells and authentic [^{3}H]PAF

[^{3}H]Acetate-labelled polar lipid, extracted from the medium fraction, which comigrated with unlabelled authentic PAF on t.l.c. plate, was extracted and mixed with appropriate amount of unsaturated PAF ($C_{18:1}$). A portion of the sample and authentic [^{3}H]PAF were separately injected onto a silica gel column and the solvent system used is described in the legend to Fig. 1. Fractions were collected every 0.2 min from 10 min to 30 min after injection and the radioactivity was determined. (●), A23187; (○), control; (△), [^{3}H]PAF standard.

1 μCi of [^{3}H]lyso-PAF and 100 μM-acetyl-CoA for 40 min were stimulated with ionophore A23187 for designated times. The lipid components were extracted and separated on t.l.c. Fig. 7 shows that after addition of 5 μM of ionophore A23187 to the cultures, the radioactivity in the PAF fraction increased rapidly from 28.6% of total lipids at 0 min (i.e. 40 min after addition of [^{3}H]lyso-PAF) to 39.5% at 5 min and 35.5% at 10 min. At incubation times beyond 10 min, the amount of PAF decreased dramatically to the control level (12.5%) at 30 min, which also was seen in the bioassay (Fig. 2), and concurrently [^{3}H]lyso-PAF increased quickly from 49.5% at 10 min to 72.3% at 30 min. In contrast, in the cells treated with DMSO alone without ionophore, the amount of PAF did not increase but decreased to 11.8% and 10.1% at 10 min and 30 min, respectively.

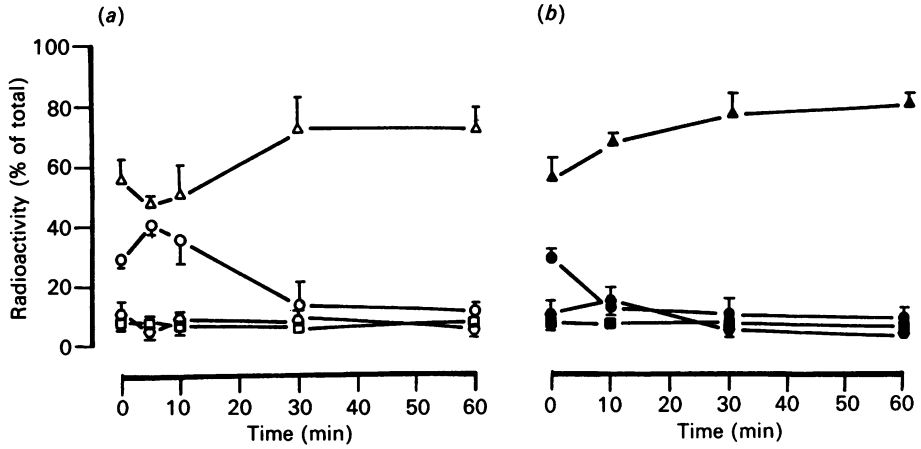

Fig. 7. Time-course of the effects of ionophore A23187 on the incorporation of [^{3}H]lyso-PAF into PAF and other lipids

The experiment was performed according to the procedure described in Materials and methods. Kupffer cells which had been challenged with [^{3}H]lyso-PAF and acetyl-CoA for 40 min were stimulated with 5 μM ionophore A23187 (*a*) or DMSO alone (*b*) for indicated time and the reaction was terminated as described. The cells and medium were extracted and the different metabolites of [^{3}H]lyso-PAF were analysed on t.l.c. Each data point is the mean value ± S.D. of measurements from triplicate plates of a single cell preparation. The radioactivity contained in the total lipids extracted from cellular and medium fractions varied in the range of 64730–68080 d.p.m. and 67726–259183 d.p.m., in the ionophore A23187-stimulated and control plates, respectively. (○ ●), PAF; (△ ▲), LPAF; (○ ●), PC; (□ ■), neutral lipids.

Table 2. Incorporation of [^{3}H]lyso-PAF into PAF and other lipids by rat Kupffer cells stimulated with ionophore A23187

The experiment was performed as described in the legend to Fig. 7 and in Materials and methods. After treatment with ionophore A23187 (5 μM) or DMSO alone Kupffer cells and medium were extracted and analysed separately on t.l.c. for PAF and other lipids. Data represent the mean values ± S.D. of measurements from triplicate plates from a single cell preparation. NL, neutral lipids.

	% of total d.p.m.			
Conditions	Lyso-PAF	PAF	PC	NL
A23187				
Released	27.3±9.1	31.8±8.4	2.3±0.3	2.3±0.5
Cell-associated	22.1±1.5	3.6±0.7	4.9±0.3	4.8±2.2
DMSO Control				
Released	27.9±13	4.4±2.3	1.1±0.5	2.3±1.6
Cell-associated	39.8±11	7.4±2.0	6.2±0.3	11.0±3.5

Further, the level of PC and neutral lipids appeared not to change throughout the incubation period, either in the ionophore A23187-stimulated or control cultures.

When the medium and the cellular fractions were analysed separately, we observed that, comparable with the [^{3}H]acetate incorporation study (Table 1), the major amount of [^{3}H]PAF derived from [^{3}H]lyso-PAF was released into the medium, which contained 0.25% bovine serum albumin (Table 2).

DISCUSSION

The present study provides the first direct evidence that rat hepatic Kupffer cells in culture synthesize and release PAF when stimulated with calcium ionophore A23187. Previous studies in this laboratory have indicated that the perfused rat liver produces PAF and exhibits an enhanced glucose output when the liver is infused with immune complexes or zymosan which are taken up by hepatic Kupffer cells [27,28]. These observations led us to consider the possibility that Kupffer cells produce PAF upon stimulation and that this capacity may be an especially important cellular response in the communication between the sinusoidal and parenchymal cells in the liver.

Previous studies have shown that several types of cells produce PAF such as blood cells (platelets, neutrophils and basophils) [4,10–16], peritoneal and alveolar macrophages [17–20], and cultured human endothelial cells [22–26]. In this study, we have examined PAF synthesis by rat Kupffer cells using a bioassay for platelet-activating activity, as well as incorporation of both [^{3}H]acetate and [^{3}H]lyso-PAF into PAF. Analysis of the time course of PAF production by rat Kupffer cells, based on both [^{3}H]serotonin release and [^{3}H]lyso-PAF incorporation, indicated that the production of PAF by Kupffer cells reaches a maximum by 10 min following stimulation and decreases thereafter. It was observed in this study that more than half of the PAF synthesized in Kupffer cells was released into the medium, similar to that reported in cultured human endothelial cells [22,26,43] and macrophages [17] and in contrast to a study reported by McIntyre *et al.* [25] using cultured human endothelial cells. An important factor affecting the synthesis and release of PAF into the medium is the concentration of protein, i.e. bovine serum albumin, in the culture medium. Ludwig *et al.* [43] reported that bovine serum albumin increased release and synthesis of PAF in polymorphonuclear leukocytes.

The [^{3}H]lyso-PAF incorporation results in the present study are interesting in that some 40 min after challenge with 1.0 μCi of [^{3}H]lyso-PAF and 100 μM acetyl-CoA,

28.6±2% of [^{3}H]lyso-PAF was converted to [^{3}H]PAF by Kupffer cells before treatment with ionophore A23187. Such a high percentage conversion of lyso-PAF to PAF might be explained as an effect of acetyl-CoA. It is thought that acetyl-CoA may enhance the formation of PAF by preventing the reacylation of lyso-PAF into its acyl metabolite, alkylacyl-GPC. This suggestion is supported by the fact that addition of oleyl-CoA prevents the increase in PAF production resulting from the presence of acetyl-CoA [20]. Addition of ionophore A23187 further increased the conversion of lyso-PAF to PAF (Fig. 7). However, following stimulation, the time interval during which PAF synthesis was stimulated was brief. Subsequently, between 10 and 30 min following stimulation, most of the PAF was deacetylated back to lyso-PAF rather than being further acylated into alkylacyl-GPC in both the ionophore A23187 treated and control cultures, suggesting the presence of acetylhydrolase activity. It is uncertain whether or not this low acylation activity is an effect of acetyl-CoA or an essential metabolic feature of rat Kupffer cells. However, the latter possibility is suggested by the fact that a low ratio of acyltransferase activity to acetylhydrolase activity also has been observed in a study of PAF metabolism in rat Kupffer cells in the absence of acetyl-CoA (W. Chao, A. Siafaka-Kapadai, D. J. Hanahan & M. S. Olson, unpublished work).

In liver, a large part of the surface of Kupffer cells, where the induction of phagocytosis occurs, is exposed to the bloodstream, and hence the microvilli of Kupffer cell surfaces intermingle with microvilli of both endothelial and parenchymal cells [44]. This type of cellular organization provides an essential histological basis for the action of Kupffer cells in the hepatic activity of PAF and for the functional interaction between Kupffer cells and other hepatic cells. The present study and the observation that heat-aggregated IgG stimulates cultured rat Kupffer cells to produce PAF (M. E. Steinhelper, W. Chao, D. J. Hanahan & M. S. Olson, unpublished work) suggests that the Kupffer cell is an important cellular component which produces and releases PAF to communicate with hepatic endothelial and parenchymal cells thus mediating the previously observed glycogenolytic response induced by this potent lipid agonist.

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