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Perspectives in Platelet-activating Factor Research

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I. Historical Background

A REACTION involving leukocytes and requiring antigen to trigger the release of histamine from rabbit platelets was reported in the sixties (24, 426, 427) and attributed to a factor actively released from the leukocytes by a calcium- and temperature-dependent process (210, 211). In 1972 and later, Benveniste et al. (31, 32, 35) described how to obtain this principle which they named platelet-activating factor (PAF), initiated its characterization, and showed that it was released from rabbit basophils by an IgE-dependent process. Because general interest was placed in eicosanoids, for many years PAF investigations were concentrated in a small number of laboratories, such as that of Benveniste for immunology; Snyder, Muirhead, and Hanahan for biochemistry; and Mangold, Hanahan, Piantodosi, and Godfroid for chemistry. However, the situation has changed radically in the past few years, particularly after the structural characterization of PAF as a phospholipase A_2 (PLA_2)-sensitive phospholipid (34, 36, 37) and its identification as 1-alkyl-2(*R*)-acetyl-glycero-3-phosphorylcholine (37, 140) (fig. 1). Even though a variety of studies were performed with native PAF (see below), investigations accelerated when synthetic preparations became available (181). Several reviews on PAF have been published (72, 220, 395, 430, 483). Five international meetings on this subject were also held recently,† and others are planned.‡

† Second International Conference on Platelet-activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, TN, Oct. 1986; "Leukotrienes and PAF-acether '85," Paris, France, Sept. 1985; First Sandoz Research Symposium, "New Horizons in Platelet-activating Factor Research," Oct. 1985; "Is There a Case for PAF-acether Antagonists," Paris, France, June 1985; and "The Promise of PAF," London, United Kingdom, Oct. 1986.

‡ "PAF and the Immune System," Paris, France, June 25–26, 1987; Satellite Meeting on "Recent Advances in Platelet-activating Factor," Brisbane, Australia, Sept. 3–5, 1987; "There Is a Case for PAF-acether Antagonists," Paris, France, Apr. 1988; and Third International Conference on PAF, Japan, 1989.

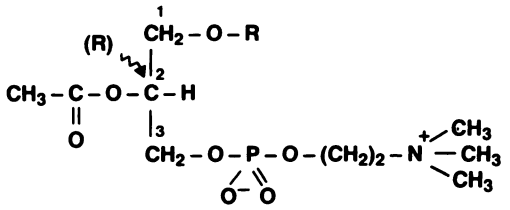


FIG. 1. Chemical structure of PAF; $R = C_{16}H_{35}$ or $C_{18}H_{37}$.

Both PAF-acether (*ace* for acetate and *ether* for the alkyl bond) and AGEPC (acetyl glycerol ether phosphoryl choline) have been used in the literature to depict PAF. In this overview of recent data, 1,-O-octadecyl(or hexadecyl)-2(*R*)-acetyl-glycero-3-phosphorylcholine (fig. 1) (see below for chemical nomenclature) will be simply named PAF.

II. Chemical Synthesis of PAF

PAF is a chiral and unsymmetrically substituted D-glycerol derivative. As an ether phospholipid, its structure is closely related to the naturally occurring plasmalogens (195). Thus, plasmalogens served as convenient chiral precursors for the preparation of PAF, especially its ^3H-labeled analog, by catalytic reduction of the C_1-vinyl ether side-chain and acetylation of the C_2-hydroxyl group.

Several total synthetic schemes for PAF with defined chain-length at C_1 have been devised. In general, the C_1-alkoxy side-chain, the C_3-phosphorylcholine moiety, and the C_2-O-acetyl group are sequentially introduced to a differentially protected chiral glycerol intermediate (215, 218, 219, 295). To avoid acetyl migration, the C_2-O-acetyl group is preferably introduced last (215). Although these reactions generally lead to good yields, the need for selective blocking and deblocking of the three hydroxyl groups often requires a lengthy (11 to 15 steps) synthesis with an overall yield of only 5 to 25%.

A key question in the total synthesis of PAF is the preparation of the chiral glycerol intermediate. The

chemistry developed in previous studies of ether lipids and some general experimental procedures have been reviewed in detail (212). The original synthesis from D-mannitol has the drawback of partial racemization during an inversion step, yielding less than 100% pure *R* enantiomer (154). Other degradative schemes using L-arabinose (246), L-ascorbic acid (245), and D and L-serine (287) as chiral starting materials were also developed. A novel enantioselective synthesis of individual enantiomers of C_{18}-PAF from D and L-tartaric acids was also described (176). Alternative ways of liberating the three hydroxyl groups in a regioselective sequence involve reductive cleavage of an acetal bond with diisobutyl aluminum hydride (451) (fig. 2) and conversion of a cyclic tin intermediate to an asymmetric C_1-hydroxyl-C_3-ester derivative (301).

Attempts to prepare optically pure PAF from synthetic starting materials have also been made. A procedure using asymmetric reduction with a complex hydride reagent (BINAL-H) of an alkoxymethyl alkenyl ketone yielded the corresponding alcohol with an optical purity of 80% (443). Ozonolysis and reduction of the alkenyl group led to alcohol with 5% optical purity, which can be converted to PAF by established routes.

III. Biochemistry of PAF

A. Introduction

The discovery of the simultaneous release from hog leukocytes of PAF and its deacetylated derivative lyso-PAF (1-alkyl-2-lyso-GPC), which was converted by chemical acetylation into a product with chromatographical and biological properties indistinguishable from those of PAF, gave the first evidence that lyso-PAF is a possible precursor and/or metabolite of PAF (375). This finding also suggested that PLA_2 activation is involved in the biosynthesis of PAF, which was confirmed with rabbit platelets, in which the inhibition of PAF and lyso-PAF formation correlated with inhibition of PLA_2 activity (34). Nevertheless, it was difficult, at the time, to establish whether lyso-PAF is a precursor or a catabolite of PAF. The studies of Wykle et al. clarified the role of lyso-PAF as the immediate precursor of PAF; an acetylation reaction catalysed by a unique acetyltransferase was described as the rate-limiting step in the formation of PAF (519). This concept has since been extended to other cell types (6, 8, 10, 112, 316, 333, 369, 394). Meanwhile, other studies demonstrated a rapid destruction of PAF in plasma (161, 364, 464) by an acid-labile factor identified now as an acetylhydrolase (161), leading to the formation of lyso-PAF as the end product. In cell systems, lyso-PAF is the obligatory intermediate in the conversion of PAF into alkylacyl-GPC [1-alkyl-2(*R*)-(long chain) acyl-GPC] by a sequential deacylation-reacylation reaction (fig. 3) (6, 8, 83, 362, 463). Stored in cellular membranes, alkylacyl-GPC is not only the end product of the cellular catabolism of PAF but also its potential precursor—via lyso-PAF—in stimulated cells (8, 463). In other words, lyso-PAF is an obligatory intermediate for both biosynthesis and inactivation of PAF in a bicyclic metabolic pathway (fig. 3).

B. Metabolic Cycle of PAF

The deacylation-reacylation cycle of PAF is accounted for by two opposing pathways (fig. 3): (*a*) biosynthesis of PAF by the sequential activities of PLA_2 and acetyl transferase which depends on cell activation and requires the presence of calcium; (*b*) inactivation and conversion of PAF into its precursor by a deacylation-reacylation reaction catalyzed by acetylhydrolase and acyltransferase. This pathway is independent of cell stimulation.

Recent reports indicate that arachidonic acid (AA) may represent one of the major fatty acids incorporated into alkylacyl-GPC during the deacylation-reacylation cycle (121, 122, 259, 294, 386, 387). In addition, preliminary experiments suggest that alkylacyl-GPC is both

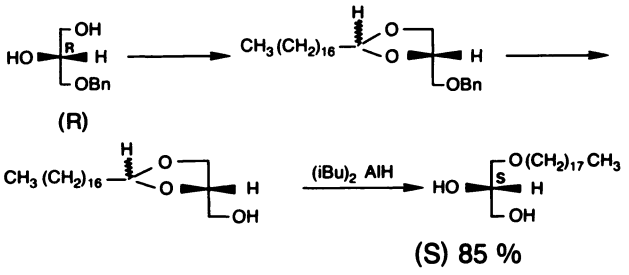


FIG. 2. Regioselective formation of chiral glycerol ether (332).

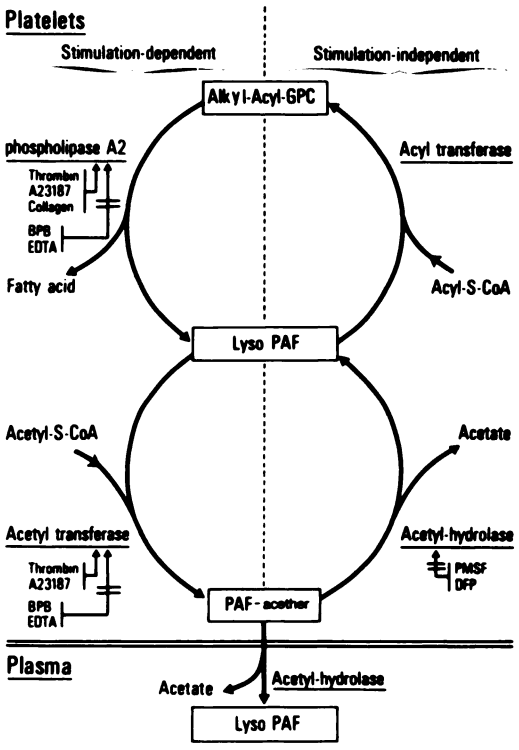


FIG. 3. The metabolic cycle of PAF in platelets and plasma.

the precursor of PAF and an important source of AA (9, 18, 115, 120, 450). Under these conditions, the deacylation-reacylation cycle may play an important role in cell regulation, activating cells releasing PAF and AA through a common mechanism (deacylation), followed by inactivation (reacylation) as cells return to their inactive state.

Most PAF metabolizing enzymes (phospholipase A_2, acetyltransferase, and acyltransferase) are located in the membranes except acetylhydrolase which is located in the cytosol (56).

1. Biosynthesis of PAF. a. PHOSPHOLIPASE A_2. The formation of PAF can be inhibited by PLA_2 inhibitors: BpB, mepacrine, 874 CB,§ and EDTA (8, 34, 88, 90). The activation of PLA_2 is calcium dependent (478) and, generally, agonists which stimulate calcium mobilization induce the formation and release of PAF (289). Contrary to other mediators, PAF is not stored in the cells but is present in the form of the inactive precursor alkylacyl-GPC linked to membrane structures (463). Upon cell stimulation, PLA_2 cleaves phospholipids at the 2(*R*) position leading to the release of fatty acids and the concomitant formation of lysophospholipid derivatives (478). A marked decrease of the cellular content of alkylacyl-GPC is accompanied by a concomitant release of lyso-PAF and PAF to the extracellular and intracellular media (8, 463). The primary production of lyso-PAF by stimulated platelets can be blocked by BpB but not by phenylmethylsulfonyl fluoride (PMSF), a potent inhibitor of PAF deacetylation. Clearly, under these conditions, lyso-PAF is the product of PLA_2 activation rather than of PAF degradation by acetylhydrolase (463).

Regarding the substrate specificity of PLA_2, choline phosphorylglyceride (CPG) is the major phospholipid hydrolyzed by PLA_2 during platelet activation (51, 247, 291, 307, 385). An exclusive release of AA from CPG has been observed with several tissues under appropriate simulations (18, 50, 221, 238). This indicates that AA linked to the 2(*R*) position of CPG is required for optimal PLA_2 activity, at least in intact cells. Previous studies have demonstrated high levels of alkylacyl-GPC in various cell types (8, 146, 325, 330, 445–447). Furthermore, investigations on the molecular compositions of phospholipids showed that alkylacyl-GPC contains a high amount of AA at the 2(*R*) position (326, 445–448), thus supporting the role of alkylacyl-GPC as a potential source of AA. Recent studies using rabbit peritoneal neutrophils (450), human polymorphonuclear leukocytes (120), and rat (9) and guinea pig (18) alveolar macrophages demonstrated that alkylacyl-GPC may represent an important source (>40%) of the total AA released by these cells during their stimulation by calcium ionophore A23187, although no preference of PLA_2 for 1-alkyl or 1-acyl species has been noted in these studies.

It is believed that glucocorticoids inhibit PLA_2 activity by inducing in target cells the synthesis and/or release of inhibitory proteins named lipocortin (147). The activity of lipocortin is dependent on its phosphorylation/dephosphorylation status (217). The existence of a protein with lipocortin-like properties was reported in rabbit platelets recently (465). In thrombin-stimulated platelets, the anti-PLA_2 activity of this protein was reduced in parallel to its phosphorylation, probably by protein kinase C (PKC). Indeed, phorbol myristate acetate (PMA), a specific activator of PKC, markedly reduced the anti-PLA_2 activity of lipocortin with its concomitant phosphorylation. This decrease in lipocortin activity was correlated with a marked potentiation by PMA of PLA_2 stimulation, i.e., AA release and lyso-PAF production induced by the calcium ionophore A23187. Similar results were obtained with other cells (190, 319, 497). Furthermore, treatment of rat peritoneal leukocytes with hydrocortisone, which induces the synthesis of lipocortin, leads to the inhibition of zymosan-induced release of lyso-PAF (359).

It thus appears that the phosphorylation of lipocortin by PKC may be a key mechanism for the regulation of PLA_2 activity and the control of PAF biosynthesis. A new endogenous inhibitor of PLA_2 was recently identified in human platelets (20), which is heat stable, resistant to trypsin, extractable by chloroform/methanol, and accordingly may be associated with lipids. This inhibitor is a mixture of unsaturated fatty acids including AA (21), which inhibit endogenous PLA_2 with a 50% inhibitory concentration (IC_{50}) of 0.5 μM. The AA released during cell stimulation may thus control PLA_2 activity, which is a new concept for the feedback regulation of this enzyme.

b. ACETYLTRANSFERASE. Lyso-PAF, an immediate precursor of PAF produced during cell stimulation, can serve as the substrate of two different pathways. It can be acylated by an acyltransferase into alkylacyl-GPC (see below) or acetylated by an acetyltransferase into PAF (fig. 3). Acetyltransferase is the limiting step for the formation of PAF and may thus have an important function in the control of inflammation. Its activation correlates with calcium influx into cells (183, 184). The distinction between acetyltransferase and long chain acyltransferase is based on their different sensitivities to detergents and on the fact that acetyl-CoA does not competitively inhibit the long chain acyltransferase (519). Potent and selective inhibitors of acetyltransferase are not yet available, and agents such as BpB and diisopropyl fluorophosphate (DFP) inhibit both acyl- and acetyl transferase (519). The conversion of lyso-PAF into alkylacyl-GPC in rabbit platelet homogenates is selectively inhibited by calcium as described previously (306). Similar findings were obtained in intact platelets (466) in which calcium influx was induced by addition of ionophore A 23187. This is in accordance with the ability of calcium ionophore A 23187 to inhibit arachidonyl transferase in the intact macrophage (260). In contrast,

§ See table 8 for chemical and/or generic names.

acetyltransferase is activated by calcium (333, 519). These observations indicate two opposing effects of calcium on PAF metabolism, inhibition of lyso-PAF reacylation and activation of lyso-PAF acetylation, which would shift lyso-PAF into the acetylation pathway and thus enhance PAF biosynthesis. In addition, the acetyl transferase activity which has been located in the intracellular membranes (6, 185a, 384) can be regulated by protein phosphorylation involving either cyclic adenosine monophosphate (cAMP)-dependent protein kinase (185) or PKC (281). The calmodulin antagonist trifluoperazine inhibits the acetylation of lyso-PAF by stimulated human neutrophils (49), but the specificity of this antagonist was ruled out (184).

Platelets produce much less PAF than lyso-PAF (1/30 to 1/100 in ratio (34, 128, 463) which may be explained by the four following possibilities.

(*a*) The major part of lyso-PAF formed by stimulated platelets is rapidly released to the extracellular medium (463) and thus escapes from acetyltransferase.

(*b*) Like other cells, platelets reacylate lyso-PAF into alkylacyl-GPC by the acyltransferase, which limits the availability of lyso-PAF for acetyltransferase.

(*c*) PAF synthesized by platelets is rapidly converted into lyso-PAF by the highly active acetylhydrolase in the cytosol (see below). Indeed, PMSF, which strongly inhibits cytosolic acetylhydrolase, favors the accumulation of PAF by human platelets (462). However, the activity of this enzyme varies considerably among cell types and animal species. The ratio between acetyltransferase and acetylhydrolase activities [which is less important in platelets than in other cells (432)] may be a determinant factor for the regulation of PAF biosynthesis. As recently reported (288), exogenous albumin can enhance the formation and release of PAF in stimulated human neutrophils, possibly through binding to PAF, and it thus prevents its catabolism by the intracellular acetylhydrolase. Accordingly, high concentrations of albumin have been shown to reduce the rate of PAF conversion in rabbit platelets (362).

(*d*) A deficiency in acetyltransferase activity might explain the low PAF formation as observed, for example, in human lymphocytes (243). We failed to demonstrate the formation of PAF by stimulated rat platelets although lyso-PAF was produced. Pretreatment of rat platelets with the acetylhydrolase inhibitor PMSF does not increase the ionophore A23187-induced PAF synthesis, indicating that degradation is not responsible for the failure to detect PAF, and that rat platelets are probably deficient in acetyltransferase.

2. Inactivation of PAF. a. ACETYLHYDROLASE. The degradation of PAF is ensured by acetylhydrolase, a highly active enzyme which converts PAF into lyso-PAF by removing the acetyl group from the 2(*R*) position (7, 70, 56, 161). This enzyme is present in the intracellular and extracellular compartments. Its intracellular form is found in the cytosolic fraction of various cells and tissues (56), whereas the extracellular form is recoverable from plasma (54, 161). The properties of the plasma enzyme are similar to those of the cytosolic enzyme except that the former is resistant to the action of proteases (54) and is resistant to serine-hydrolase inhibitors PMSF and DFP. It was proposed that intracellular acetylhydrolase may undergo modification such as glycosylation to facilitate its secretion into the vascular compartment (432).

In contrast to PLA_2, acetylhydrolase cleaves only the short chain fatty acids esterified at the 2(*R*) position of phospholipids and is calcium independent. In the studies using platelets from different species (rabbit, human, and rat), a positive correlation was observed between the aggregability of these cells in the presence of PAF and their capacity to hydrolyze it. This probably indicates that the more the cells are sensitive to PAF, the more they need to inactivate it (L. Touqui, unpublished).

b. ACYLTRANSFERASE. Whatever its route of formation, lyso-PAF is cytotoxic [lytic and detergent properties (507)]. Its elimination is achieved by an acylation system which introduces a long chain fatty acid into the 2(*R*) position of lyso-PAF (fig. 3); the resulting alkylacyl-GPC (8, 83, 121, 122, 259, 294, 306, 386, 387, 463) then becomes an integral part of the membrane (463). Exogenous lyso-PAF is principally converted to alkylacyl-GPC, whereas a relatively minor amount is converted to PAF. Thus suggests that acyltransferase has a higher affinity for lyso-PAF and/or greater rate of reaction than acetyltransferase. AA is one of the major fatty acids incorporated into lyso-PAF (122, 259, 294, 387) by this system which is catalyzed mainly by CoA-independent transacylase using phosphatidylcholine (PC) as the source of AA (259, 294, 386). Free AA is initially incorporated into PC by a CoA-dependent acyltransferase (308) and thereafter transferred to lyso-PAF and other ether lipids by a CoA-independent transacylation (129, 130, 258, 294, 444). The reacylation of lyso-PAF is inhibited by Ca^{2+} with an IC_{50} of 50 to 100 μM, suggesting that during cell activation a rise in Ca^{2+} influx may inhibit this enzyme (fig. 4), leading to a transient accumulation of lyso-PAF which favors its utilization by acetyltransferase for PAF synthesis (466).

3. Metabolism of PAF in vivo. Administration of [^{3}H]PAF i.v. is followed by its rapid clearance from blood ($t_{1/2}$, 30 s) with a parallel increase of radioactivity in various organs and tissues (53, 267). Chromatographic analysis of the tissue extracts showed that part of PAF is converted into alkylacyl-GPC and lyso-PAF, indicating that the deacylation-reacylation process may also occur in vivo. Ten min after administration of labeled PAF, a high proportion of the radioactivity taken up by tissues is still found as intact PAF despite the presence in plasma of acetylhydrolase (161, 364).

PAF causes a platelet-independent bronchoconstriction of the blood-free perfused guinea pig lung (277), but fails to induce a similar effect when administered i.v. to platelet-depleted guinea pigs (see below). A rapid inac-

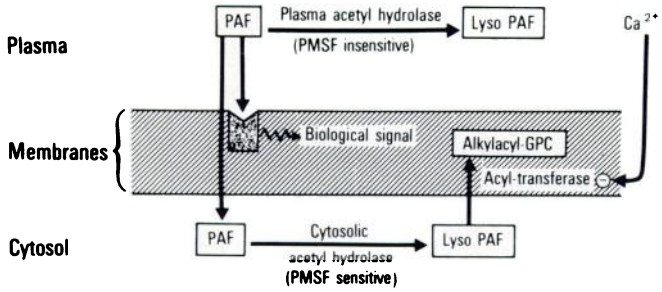


FIG. 4. A schematic diagram showing the location of different enzymes involved in the inactivation of PAF in both the extracellular and intracellular compartments. PAF interacts with its receptor to induce a biological response, initially phosphoinositide breakdown. After crossing the membrane, PAF is deacetylated by cytosolic acetylhydrolase. PAF is stored in the membrane in the form of its precursor, alkylacyl-GPC. Reprinted by permission from *Biochemical Journal* **242:** 555–560, 1987, The Biochemical Society, London.

tivation of PAF by plasma acetylhydrolase probably prevents its access to the lung. In this respect, it was suggested that the short duration of the hypotension induced by low doses of PAF (57) may be due to the rapid degradation and/or clearance of PAF from the blood (53).

C. Alternative Pathways

1. CDP-choline cholinephosphotransferase. The biosynthesis of PAF by the transfer of a phosphocholine group into 1-alkyl-2(*R*)-acetyl-glycerol has been characterized in numerous tissues, such as rat spleen (383), human neutrophils (10), and rabbit platelets (52). This reaction is catalyzed by a specific CDP-choline cholinephosphotransferase different from that involved in the conversion of diacylglycerol into PC (383). The route of formation of the substrate alkylacetylglycerol, which could be the limiting factor of this pathway, has been recently established in rat spleen (274). Possibly, the antihypertensive activity observed after the i.v. injection of alkylacetylglycerol may be due to its in vivo conversion into PAF (53, 55, 327). The cholinephosphotransferase activity appears to be independent of cell stimulation (10, 431) and may occur in the absence of added calcium (404). The physiological significance of this pathway remains unclear.

2. Methyl transferase. PC can be derived from phosphatidylethanolamine (PE) by two successive methylations catalyzed by methyl transferases using S-adenosylmethionine as methyl donor (reviewed in ref. 380). In theory, inhibition of the alkylacylglycerophosphoethanolamine (GPE) methylation can reduce the cell capacity to produce PAF. However, the cellular level of alkylacyl-GPC is much higher than that required physiologically, and it is unlikely to be a limiting factor in PAF biosynthesis. Indeed, it has been shown that the biosynthesis of PAF can be dissociated from phospholipid methylation in thrombin-stimulated rabbit platelets (380, 461). This correlates with a previous report that platelet activation and phospholipid methylation are completely dissociated in rat platelets (379). Furthermore, no significant incorporation of methyl group into PAF or lyso-PAF was detected when these lipids were extracted from [^{3}H]methionine-labeled and thrombin-stimulated platelets (380). Thus, the formation of PAF by direct methylation of alkylacetyl-GPE is unlikely to occur in the cells.

Interestingly, methyl transferase inhibitors, such as 3-deazaadenosine and L-homocysteine, inhibit the formation of PAF by mechanisms other than the inhibition of phospholipid methylations (461), probably by interfering with the methylation of some proteins involved in the biosynthesis of PAF.

D. Stimulus-Response Coupling

The primary biological signal induced by PAF is certainly the increase of phosphatidylinositol (PI) breakdown and the subsequent formation of diacylglycerol (DAG) (303), one of the important intracellular second messengers. DAG is a potent activator of PKC (336) and may thus control various cell functions. On the other hand, PI breakdown generates inositol 1,4,5,-triphosphate (IP_3) which is involved in the intracellular calcium mobilization (40). Thus, activation of PKC and calcium influx may act synergistically to amplify the biological signal of PAF.

An increase of PI turnover and intracellular level of calcium are thought to be the major events linking receptor occupancy to the cellular response. In contrast, cAMP appears to be the main inhibitory mediator. As a rise of the intracellular level of cAMP inhibits all cellular responses, it has been suggested that the reverse, i.e., inhibition of adenylate cyclase, may cause cellular activation. In this respect, it has been shown that PAF can suppress a rise of cAMP (203).

IV. Structure-Activity in PAF Series

PAF analogs have been synthesized in order to: (*a*) establish the structural requirements for activity; (*b*) search for new antagonists; and (*c*) achieve possible therapeutic effects, such as selective antihypertensive activity, and eliminate undesirable actions, such as anaphylaxis. A large number of PAF analogs have been synthesized by varying the substituents of the glyceryl backbone.

The effects of these various changes on rabbit platelet aggregation are summarized as follows.

A. Chirality of C_2

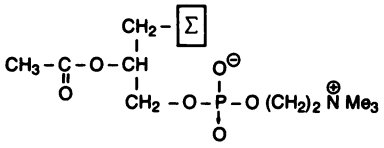
PAF has an *R* configuration at C_2. Reversion of the chirality (*S* isomer, 1) leads to a very significant decrease of the activity of PAF (214, 215). The 50% effective concentrations (ED_{50}) of isomers are: $R = 5.7 \times 10^{-11}$ M; $S = 1.7 \times 10^{-7}$ M; and racemic $= 2.2 \times 10^{-10}$ M.

B. Changes in the Substitution of the Glyceryl Backbone

1. Change in C_1. Table 1 shows that the ether function is an absolute requirement for the biological activity.

TABLE 1

Structure-activity relationships in PAF series: influence of the deletion of C_1 ether function on rabbit platelet aggregation. Figures for intensity of aggregation (PAF = 100%).



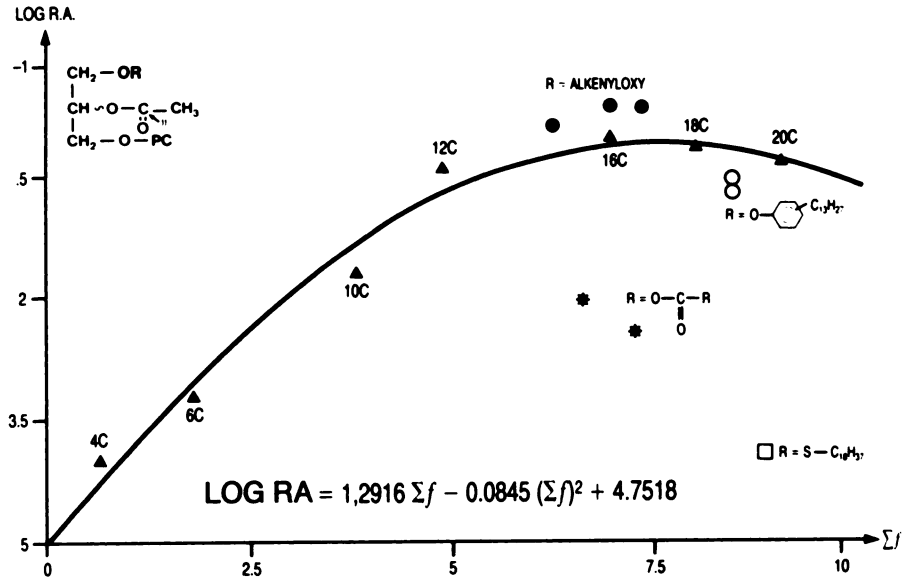
Σ	PLATELET AGGREGATION STIMULATION	
	PRP	WP
$-S-C_{18}H_{37}$	0.02	—
$-O-\overset{O}{\overset{\|\|}{C}}-C_{15}H_{31}$	—	0.25
$-O-\overset{O}{\overset{\|\|}{C}}-C_{17}H_{35}$	—	0.10
$CH_2(CH_2)_nCH_3$		
n = 14	0.50	—
n = 15	0.75	—
n = 16	0.44	—
n = 17	—	<0.01
$-O-\overset{O}{\overset{\|\|}{C}}-NH-R$	inactive	inactive

* PRP, platelet-rich plasma; WP, washed platelets.

Indeed, the replacement of oxygen by isoteric groups such as sulfur (316), methylene (76, 454, 518), ester (454), or various nitrogen radicals leads to an almost complete disappearance of agonistic effect. The biological activity of the 1-thioether-2-acetamido analog of PAF, a combination of two isosteric modifications, has not been reported (178). It is of interest to note that the 1-S-hexadecyl-2-O-ethyl analog of PAF inhibits the growth of HL-60 leukemic cells and human ovarian carcinoma cell liners at 1 μg/ml (323).

Fig. 5 shows the effect of varying the length of the alkyl chain (and the consequent lipophilicity). It can be seen that maximum activity for platelet aggregation and hypotension were observed with C_{16}–C_{18} analogs (corresponding to a hydrophobic fragmental constant (382), $\Sigma fc = 7.5$) whereas the maximum bronchoconstrictive effect was found with the shorter C_{14} chain ($\Sigma fc = 5.90$) (180). All agonistic activities in these series are closely correlated. Furthermore, results obtained with several 1-alkyl-phenoxy analogs of PAF clearly showed that the position of the fatty chain with respect to the glyceryl backbone is also important for activity.

Compared to PAF, the meta analog exhibited a similar activity, and the para analog was weakly active, whereas the ortho isomer was totally inactive (518). Regarding the degree of saturation, the presence of one or two double bonds in the chain slightly reinforced the agonistic activity of 1-O-octadecyl analogs, e.g., [18:2] > [18:1] > [18:0] (449). Multiple oxygen substitution of the al-



RA = Relative Activity in comparison with C_{18} PAF vs washed rabbit platelet aggregation

FIG. 5. Agonistic activity (in vitro and in vivo) as a function of the length of the alkyl chain on C_1. (1) All products are racemic. (2) Σfc: sum of the hydrophobic fragmental constants (fc) calculated from the lypophilicity table (318); maximal activity occurs when $6 < \Sigma fc < 8$. (3) Agonistic activity expressed as log (effective concentration). (4) Parabolic equation between various agonistic activities and hydrophobicity of the alkoxy chain in position 1 has been calculated (courtesy of Prof. J. J. Godfroid, ref 180):

$$\text{Agonistic activity} = a + b\,\Sigma fc - c(\Sigma fc)^2)$$

with WP: $a = 3.226$, $b = 1.049$, $c = 0.071$; PRP: $a = 4.750$, $b = 1.284$, $c = 0.084$; hypotension: $a = 4.785$, $b = 1.236$, $c = 0.088$; bronchoconstriction: $a = 4.912$, $b = 1.804$, $c = 0.153$.

koxy chain resulted in a significant decrease of biological responses (515).

2. Change in C_2. Interest in modifying the ester function in C_2 was prompted by the observation that acetylhydrolase transforms PAF into the inactive metabolite lyso-PAF by removing the acetyl group from the 2(*R*) position (432). Various modifications of the C_2 ester function, e.g. methylcarbamate, do not result in an increase in potency (189) (cf. fig. 6) but significantly increase the serum half-life for these PAF isosteres (>1.7 min, half-life of the C_{16} PAF) (189). Although the propionyl homologous is nearly as active as PAF (454), the activity decreases rapidly as the size of the acyl group is increased.

Surprisingly, the C_2-ethoxy analog retained ~4% of the PAF activity (189, 295, 339). The residual PAF-like activity of this isostere demonstrated that no in situ transfer of the labile acetyl group is required for the actions of PAF, which was confirmed by the activity of both the nitrate isostere (189) (only 15 times less active than the acetyl ester) and the n-Pr analog (520) (80 times less active). Replacement of the 2-acyl moiety with fluorine or chloride also reduced proaggregatory activity significantly (353). Therefore, the requirement for effectiveness seems to be mainly related to the length and the bulk of the C_2 substituent, the maximal activity being observed for substituents with a length 6–7 Å.

3. Change in C_3. Table 2 shows that replacement of the phosphoryl group by a phosphonate function does not modify the activity considerably (324, 515). However, deletion or replacement by an ethoxide (517), a carboxyl (509), or a methylene sulfonyl methyl moiety (514) reduces significantly or abrogates the platelet-stimulatory activity.

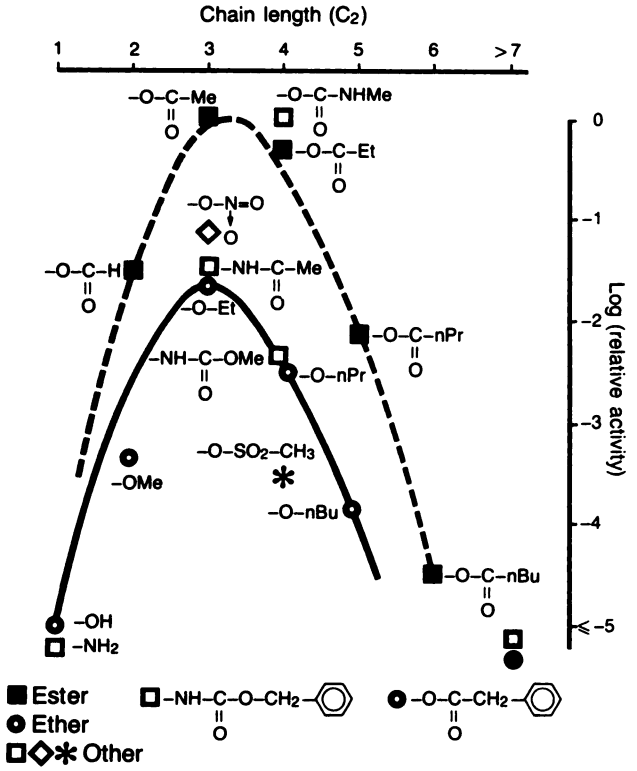
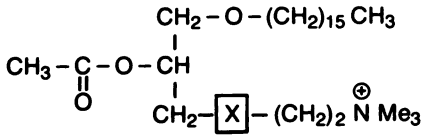
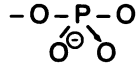
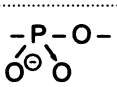
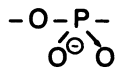


FIG. 6. Structure-activity relationship in PAF series: influence of the change in the nature of the substitution on C_2 with respect to rabbit platelet aggregation (modified from ref. 70).

TABLE 2

Structure-activity relationships in PAF series: influence of the modification or deletion of phosphoryl group situated on C_3. See table 1 for explanations.

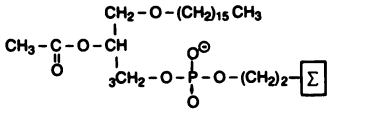


X	PLATELET AGGREGATION STIMULATION (PRP)
	100
-O-	0.01
	20.83
	11.36

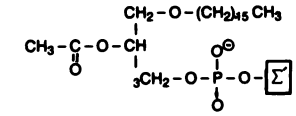
Regarding the quaternary polar head group, replacement of the trimethylammonium portion of choline with other nitrogen-containing moieties has led to analogs with potent proaggregating activities (see table 3 for details) (126, 341). In the choline series, the activity decreases significantly in the following order: trimethylammonium > dimethylamino > methylamino > amino group (126). Among the numerous PAF isosteres reported, it is remarkable that those in which the quaternary ammonium group was replaced by several cyclic derivatives (such as N-methyl piperidinium, N-methyl pyrrolidinium, and N-methyl morpholinium) are even more active than PAF itself (see table 3) (126). The distance between the phosphoryl group and the positive polar head is also critical: increasing the length of this bridge results in a gradual but progressive decrease in the hypotensive and platelet aggregation responses (460, 517). Interestingly, the hexyl (U 66985) or the decyl (U 66982) compounds were reported to have no agonistic activity but inhibit PAF-induced platelet aggregation (460). Similar findings have been demonstrated by substituting the choline chain with various phenyl-containing groups (517). Analogs in which the phosphocholine moiety has been substituted with a methyl group have been recently prepared (517). With respect to both the blood pressure and platelet aggregation responses, it is interesting to note that substitution at the alpha position of the nitrogen group significantly enhanced activity in comparison with natural PAF. In contrast, such substi-

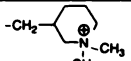
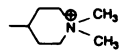
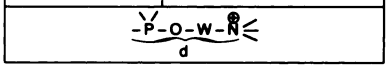
TABLE 3

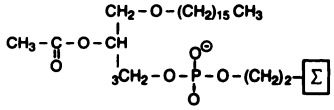
Structure-activity relationships in PAF series: influence of the modifications in the nature of the polar head situated on C_3. See table 1 for explanations.



Σ	PLATELET AGGREGATION STIMULATION (PRP)
	539.22
	44.27
	630.20
	55.00
	120.65
	0.47



Σ′	PLATELET AGGREGATION STIMULATION (PRP)
	113.99
	11.17
	



Σ	PLATELET AGGREGATION STIMULATION (PRP)
$-NH_2$	0.15
$-N\,Me_2$	47.62
$-\oplus N\,Me_3$	100.00
$-N\,Et_2$	111.10
$-\oplus N\,Et_2\,Me$	200.00
$-\oplus N\,Et_3$	1.00

tution in the adjacent position to the phosphate moiety only results in little change in the biological responses (517).

C. Position Isomerism

Several investigators have reported the synthesis of positional isomers (C_1/C_2 and C_2/C_3) and the enantiomers (218, 219). It might be expected that the spatial arrangement of the three glyceryl substituents in PAF would be critical for activity. However, this was only partially the case, since only minor overall structural differences exist between the different position isomers. Indeed, only the chirality of the asymmetric center must be taken into account for activity. It may be noted that the stereochemistry of (*S*) enantiomers of the two positional isomers is similar to that of the (*R*) PAF. The loss of activity of both C_1/C_2 and C_2/C_3 (*S*) isomers may be related to the increase in the length of the substituent at position C_2 as discussed above.

D. Replacement of the Glyceryl Backbone

An analog of PAF incorporating an additional acetoxymethylene unit into the glycerol framework has been shown to be a weak stimulant of platelet aggregation (11). Wissner et al. (516) observed that racemic analogs of PAF containing a methylene group between C_2 and C_3 or between C_1 and C_2 have low activities. Furthermore, compounds in which C_1 or C_3 is substituted with one or two methyl groups are also less potent than natural C_{16} PAF. These results demonstrate that the length of the glyceryl backbone is also a key point for activity.

A closer examination of the *S* and *R* stereo isomers of C_1 or C_3 methyl homologs of PAF revealed that 1-(*S*)-methyl-PAF is a selective agonist, possessing stronger antihypertensive activity than PAF by p.o. dose with lower platelet activation characteristics (342).

V. Pharmacology and Biochemical Pharmacology

A. Systemic Effects of PAF and Analogs

The i.v. injections of PAF to different animal species is followed by marked systemic effects: hypotension (rats, baboons, guinea pigs, dogs, rabbits) (141, 192, 309–313, 481, 487); pulmonary hypertension (rabbits) (310); increased resistance of airways, i.e., bronchoconstriction [guinea pigs (68, 470, 481, 486–491), baboons (141)]; increased vascular permeability (rats, guinea pigs) (75, 199); thrombocytopenia (80, 365, 487); neutropenia; and death. Similar systemic effects are likely to occur in mice, which are killed upon i.v. PAF injections (329).

Even though PAF does not activate rat platelets in vitro, in vivo thrombocytopenia accompanies hypotension, increased vasopermeation, and a paradoxical leukocytosis (297).

B. Species and Route Dependency

The effects of PAF are markedly dependent upon the animal species and the administration route (489). For instance, when PAF is aerosolized to guinea pigs, bronchoconstriction, which is markedly tachyphylactic and blocked by aspirin (277), develops slowly; the accompa-

nying hypotension is aspirin resistant and may be due to PAF leakage to the periphery. The effects of PAF appear to depend markedly on the cell population which is reached as a first hit: circulating cells upon i.v. administration and cells located on the bronchi and/or alveoli when aerosol treatment is performed. As discussed below, it is likely that PAF interacts with the alveolar macrophages when given through the airways. Bonnet et al. (59) demonstrated that, under specific conditions, aspirin may reduce bronchoconstriction due to i.v. PAF, possibly by blocking AA metabolism. A lipoxygenase dependence of PAF effect may operate in mice, since both the peptido-leukotriene antagonist compound FPL 55712 as well as lipoxygenase inhibitors protected against PAF-induced death (521).

The mechanism of the systemic effects of PAF was studied mostly in guinea pigs and rabbits and has not been fully elucidated.

Bronchoconstriction by PAF injected i.v. at 20 to 60 ng/kg to guinea pigs is accompanied by hypotension, thrombocytopenia (487), leukopenia (predominantly neutropenia), and increased vasopermeability (199). Bronchoconstriction depends on platelet participation, most probably upon secretion of mediators which are cyclooxygenase independent (487), whereas vasopermeation (systemic extravasation observed as increased hematocrit) is at least partially mediated by leukocytes (199). In contrast, Wedmore and Williams (504) demonstrated that skin responses to PAF alone or associated with prostaglandin E_2 (PGE_2) can be elicited in rabbits depleted from circulating polymorphonuclear leukocytes. PAF displays systemic effects which are independent from circulating cells, as demonstrated by the fact that i.v. injections of PAF to neutrophil-depleted rats is followed by the release of lysosomal hydrolases into the circulation (150, 173).

Furthermore, PAF increases vascular permeability and induces systemic hypotension in rats, even though their platelets are unresponsive to PAF (399). In the guinea pig, the combined intradermal injections of PAF and PGE_1 enhance plasma protein extravasation, but prostaglandins reduce the number of platelets accumulating in situ (320).

Platelets are directly involved with PAF-induced bronchoconstriction upon its i.v. administration to guinea pigs. Not only is bronchoconstriction suppressed by immune platelet depletion or by the infusion of prostacyclin but it has been shown that, 3 min after the i.v. injection of PAF, the early intravascular platelet aggregation and neutrophil margination are accompanied by platelet diapedesis and, as a consequence, by the presence of degranulated (activated) platelets outside the vessels, in the proximity of the smooth muscle cells (fig. 7) (279, 280). Given to rabbits, PAF induces hypotension, thrombocytopenia, neutropenia, bradycardia, and release of platelet factor 4 and thromboxane (80, 192, 309–312, 364). Immediately after its infusion to rabbits, platelets and neutrophils aggregate throughout the pulmonary microvessels, and small muscular arteries and bronchioles contract.

Platelet aggregates are less prominent after 5 min, but neutrophils persist, and mononucleated cells and damaged endothelium are apparent. Within 1 h, platelet aggregates and constricted smooth muscle disappear, whereas neutrophilic and monocytic infiltration persist. The lung mechanical impairments due to PAF in rabbits are suppressed by platelet depletion, whereas the ventilatory and circulatory impairments are unaffected (193, 365).

Cyclooxygenase-dependent mechanisms may account more for death by PAF in the rabbit than in the guinea pig, since cyclooxygenase and thromboxane synthetase inhibitors were protective for the former (312), whereas aspirin or indomethacin alone is inactive for the latter (489–491).

The intratracheal instillation of PAF into rabbits induces lung inflammation, with accumulation of macrophages in the alveolar spaces and an increase of polymorphonuclear leukocytes and platelets in the alveolar capillary lumens with degenerative changes of the endothelium, highlighting the possibility that, during an inflammatory reaction, an intraalveolar release of PAF contributes to injury (93). In dogs and rats, PAF also induces a shock-like condition (23, 43, 44, 72, 84).

C. Effects of PAF on Perfused Organs and Isolated Tissues

Isolated organs or tissues are used to clarify the complexities of the effects of mediators in vivo. When added directly to the guinea pig ileum, PAF induces a slowly developing and sustained contraction that is not inhibited by antagonists of unrelated autacoids (143, 166, 469). This profile differs from that of complement components C5a and C3a, which induce histamine-dependent rapid and transient contractions. The ileum can be desensitized to PAF and cross-desensitized with its C_2-ethyl analog and, as expected, there is no cross-desensitization with C5a and C3a (437) as also noted for rabbit lung strips (92). Similarly, rabbit basophils stimulated with rabbit, human, and pig C5a do not release PAF, whereas rabbit mast cells respond to C5a with release of histamine and PAF (91).

PAF does not stimulate the isolated trachea, which may even be relaxed via a prostaglandin-independent mechanism (377). In contrast, PAF contracts the isolated rat vein (23, 403) and the guinea pig parenchyma lung strip (143, 277, 438, 469, 470). These contractions are platelet independent, long lasting (>1 h), accompanied by the formation of thromboxane B_2 and, most importantly, are markedly tachyphylactic. The effects of PAF on guinea pig parenchyma lung strips are not inhibited by the antihistamine mepyramine, by the antiserotonin methylsergide, or by cyclooxygenase inhibitors either alone or in combination, despite the suppression of the

formation of cyclooxygenase metabolites (143, 438, 469). Stimler-Gerard et al. (439) recently showed that atropine reduces the contractile effects of PAF on guinea pig parenchyma lung strips. In accordance with the concept that the in vivo and in vitro effects of PAF in the guinea pig are accounted for by different mechanisms, lung strips collected from animals given injections repeatedly with PAF i.v. still contract when exposed to PAF in vitro. The situation differs in rabbits, since i.v. infusions of PAF cause desensitization and abolish subsequent contractions of the parenchyma lung strip (92) in vitro.

The situation is still different when PAF is infused to isolated guinea pig lungs perfused with cell-free solutions (193, 277). Indeed, the airways and arterial pressures are markedly increased, and thromboxane and prostacyclin are produced. Indomethacin, aspirin, and PAF antagonists, e.g., 48740 RP, BN 52021, brotizolam, Ro 19-3704, and WEB 2086 (see below), suppress these effects of PAF. Bronchoconstriction and the formation of thromboxane are not inhibited by the antagonist of peptidoleukotrienes compound FPL 55712 at concentrations which suppress the effects of LTD_4 (277), indicating that different lung sites are involved with release of thromboxane triggered by peptidoleukotrienes and by PAF. In rat lungs, PAF causes vasoconstriction and edema, accompanied by the release of peptidoleukotrienes. Here, indomethacin is inactive against PAF, whereas the lipoxygenase inhibitor diethylcarbamazine is effective (496). Finally, PAF is relatively inactive when infused intraarterially to isolated rabbit lungs (16), unless platelets (human) are introduced into the perfusion fluid, in which case pulmonary hypertension and hydrostatic lung edema are observed (204). Thromboxane produced by the added platelets may be responsible for these effects, since the (unspecific) thromboxane synthetase inhibitor imidazole and the thromboxane antagonist 13-azaprostanoic acid reduced hypertension and suppressed edema (206).

Since early platelet aggregation and smooth muscle construction vanish 1 h after i.v. PAF in the rabbit, whereas neutrophils and monocytes persist (312), these results confirm that PAF has indirect effects, involving secondary mediators such as thromboxane, but they are far from proving that only platelets are involved in vivo. Finally, PAF perfused into the isolated guinea pig heart induces arrhythmias and decreases coronary flow and contractile force (23, 33, 253, 282, 283, 366, 456). Here again, there is evidence for lipoxygenase mediation (282).

D. Cell Sources—Cellular Effects of PAF

1. Platelets. Chignard et al. (117, 118) demonstrated that PAF is released from platelets stimulated with the ionophore A 23187, thrombin, or collagen and suggested that PAF mediates the "third pathway of aggregation," since neither its production nor its effects are blocked by aspirin or by adenosyl diphosphate (ADP) scavengers. PAF is also released from human platelets stimulated with thrombin or ionophore A 23187 (116–118, 462). However, the existence of such a PAF-mediated third pathway of platelet aggregation is a matter of controversy (cf. section VI A). PAF was discovered as a platelet-

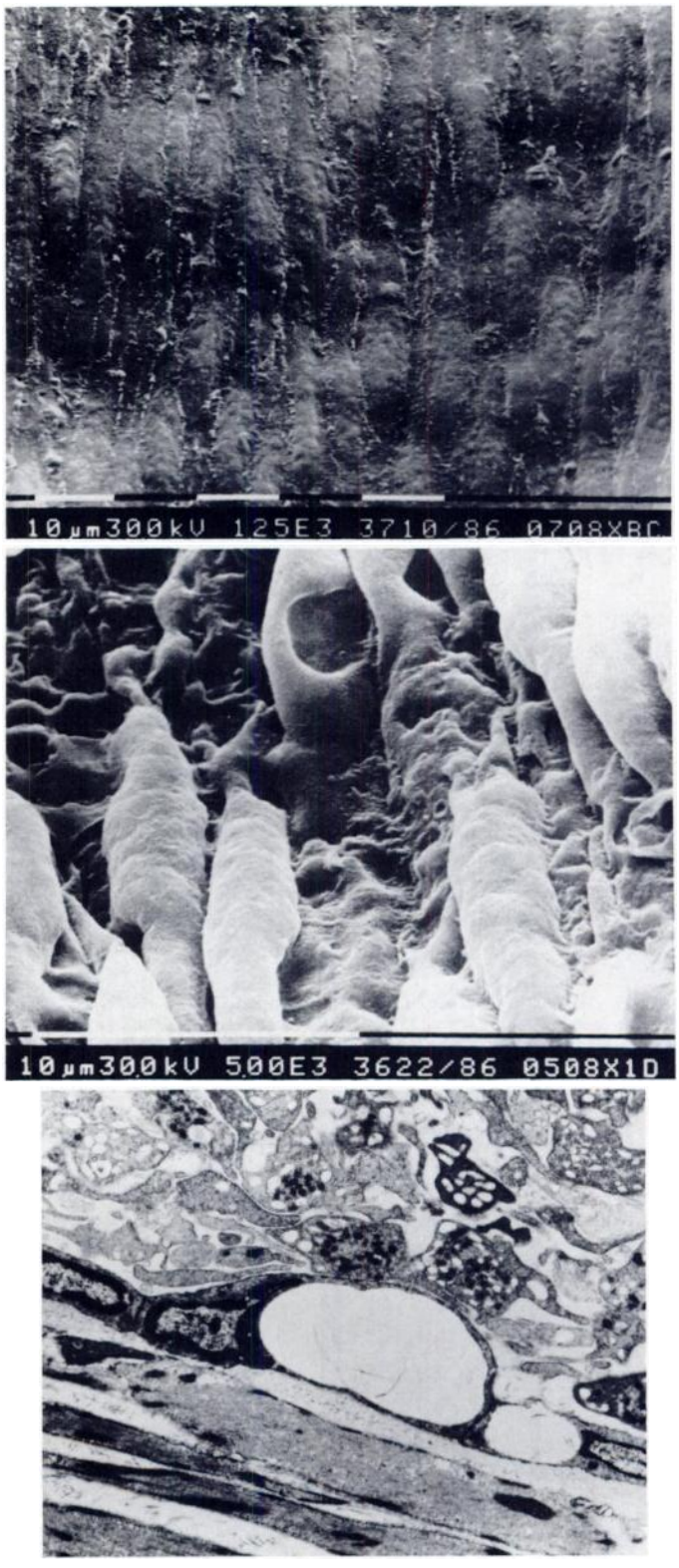


FIG. 7. *A.* Control electron micrograph of the exposed mesenteric artery before (*top*) and after (*center*) a 10-min superfusion of PAF. Note blebs on endothelial cells, magnified on the *bottom* micrograph.

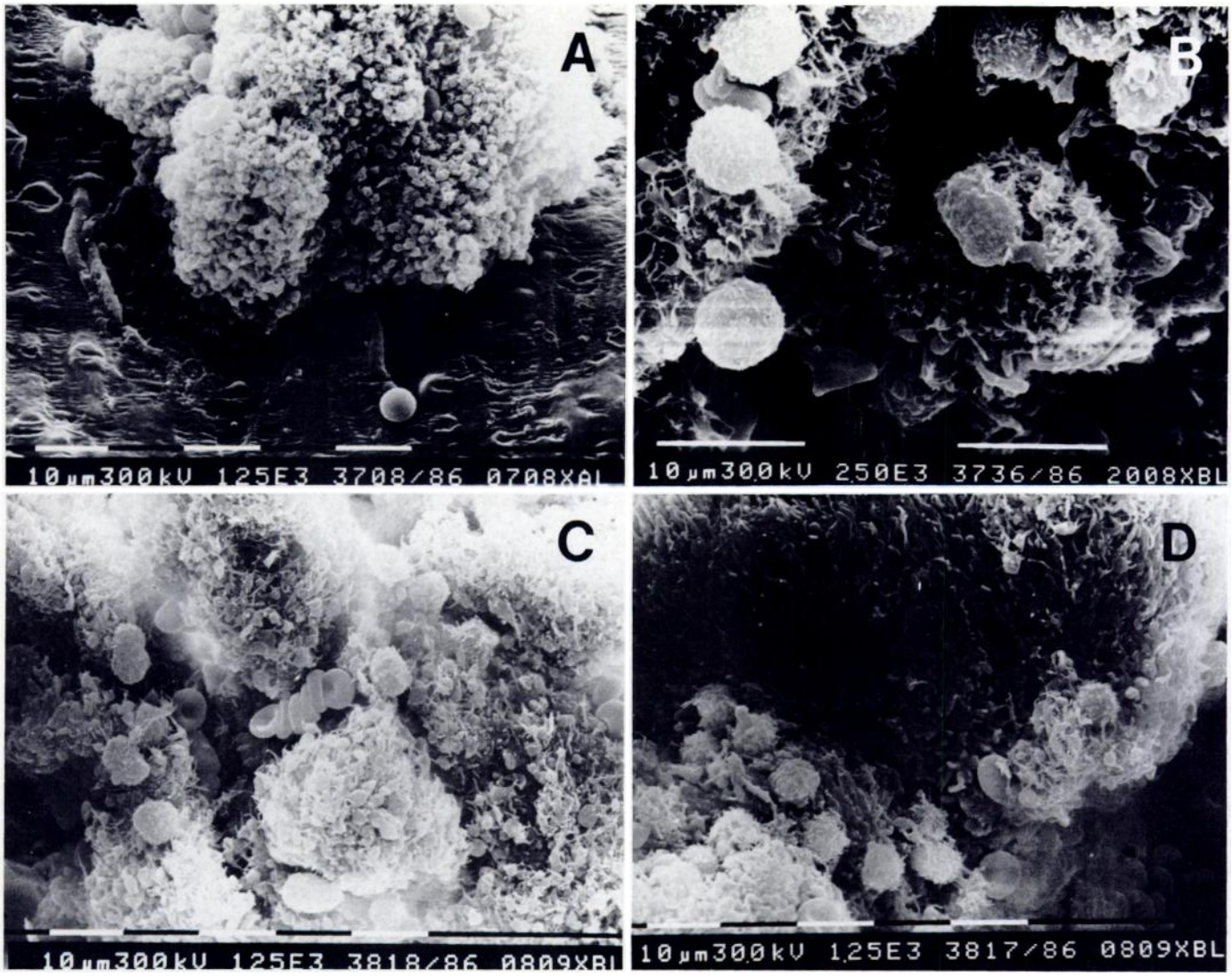


FIG. 7. *B.* Five (*A*), 9 (*B*), and 20 (*C* and *D*) min-old thrombus due to the superfusion of PAF onto the exposed mesentery artery, respectively, *left-hand*, *center*, and *right-hand* micrographs. No leukocytes after 5-min superfusion, and a large number of invading leukocytes are observed after 9 min. The 20-min-old thrombus tends to become occlusive. Figures kindly provided by Prof. R. Bourgain, Free University of Brussels.

activating agent and, indeed, it activates platelets of most animal species in vivo or in vitro, in whole blood, in platelet-rich plasma, or as isolated cells. Fibrinogen is needed for activation of human platelets, and even though the relative contribution of the arachidonate cyclooxygenase pathway and of endogenous ADP may vary, in general, PAF has its own intrinsic effect, since it activates aspirinated and ADP-deprived platelets (175, 485). Rat platelets are refractory to PAF in vitro, but high in vivo doses induce thrombocytopenia and leukocytosis (297) (cf. section VI A). In the in vivo situation, platelets may react to PAF provided by surrounding cells, such as the leukocytes. Platelets lack significant amounts of acetyltransferase, and lyso-PAF formed during stimulation with thrombin or other agents (see section III B 1 b) will only be converted into PAF itself in the presence of cell populations such as leukocytes, i.e., in mixed populations in vivo or in whole blood (127a). Platelets may thus behave as lyso-PAF donors during cell cooperation and, in turn, react to the PAF formed in their vicinity.

2. Leukocytes. PAF and lyso-PAF are released from stimulated animal or human polymorphonuclear leukocytes (PMNLs) (275, 288–290, 398).

Monocytes (90), macrophages (8, 15, 89, 314–316), mast cells (315), and eosinophils (273, 275) also release PAF-acether upon stimulation with the Ca^{2+} ionophore A 23187, zymosan particles, or IgE.

Rabbit neutrophils stimulated with *f*-methionyl-leucyl-phenylalanine are known to release different intracellular components or metabolites, including PAF. If platelets are present, the released PAF will trigger aggregation and secretion (127a, 127b). In contrast, human neutrophils seem to release also a previously unrecognized protein, named neutrophilin (118a). PAF induces leukocyte activation, with in vivo leukopenia (191–193, 297) and cell margination. In vitro activation leads to enzyme secretion and formation of superoxide and of

arachidonate metabolites (244, 340, 416). Rat neutrophils collected from pleural exudates (78a) and guinea pig alveolar macrophages (296) are activated by and desensitized to PAF; there is no cross-desensitization with *f*-methionyl-leucyl-phenylalanine. Activation of guinea pig alveolar macrophages includes arachidonate release, formation of its metabolites, reduction of the increased cyclic AMP content after exposure to betamimetics and to PGE_2, and superoxide ion formation. These effects are suppressed by different PAF antagonists, which do not interfere with *f*-methionyl-leucyl-phenylalanine (298) (Bachelet et al., in preparation).

3. Endothelial cells. Stimulated with thrombin or with an anti-factor VIII antiserum, human endothelial cells in culture release PAF (87, 88, 378). In the rabbit, i.v. injection of an anticonverting enzyme antiserum induces pulmonary lesions accompanied by neutrophil margination and associated with the appearance of PAF in the plasma. The converting enzyme being a marker for endothelial cells, this shows that PAF can indeed be released in vivo from this cell type. Interleukin-1 (IL-1) also induces the release of prostacyclin (392) and PAF (79a) from human endothelial cell cultures.

E. Direct and Indirect Effects of Systemic PAF

In both rabbits and guinea pigs, systemic PAF induces lung injury and, as a consequence, immediate and delayed effects. When PAF is infused to the guinea pig, platelet and leukocyte counts drop approximately at the same time, the latter more intensively than the former. Bronchoconstriction may be absent, according to the rate of infusion of PAF, and hypotension then increases, possibly because the reduced bronchoconstriction is unaccompanied by the compensatory hypertension usually due to the catecholamines secreted as a reaction against asphyxia.

Differences between rabbits and guinea pigs are due to the overdevelopment of arteriolar and bronchial smooth muscle, respectively, and may also involve the species-dependent formation and/or release of other mediators, likely to amplify the primary effects of PAF itself.

An important point concerns the actual role of monocyte lung infiltration and of alveolar macrophages, particularly in view of the monocyte accumulation when PAF is instilled into the rabbit trachea (93) and the accumulation of alveolar macrophages when PAF is aerosolized to guinea pigs (279, 280). Furthermore, as indicated above, PAF aerosolization into the guinea pig lung is followed by aspirin-sensitive bronchoconstriction (277). Alveolar macrophages are reached during aerosolization, since they are desensitized to further in vitro stimulation (296).

The interactions between PAF and the various protease systems involved in inflammation and shock (kallikrein, complement, fibrinolysis, coagulation) have been poorly investigated, even though these systems may be of vital importance. Emeis and Kluft (157) have demonstrated that PAF (as well as other mediators) induces the secretion of tissue-type plasminogen from rat vessel wall, which was suppressed by phospholipase and lipoxygenase inhibitors, under conditons where cyclooxygenase inhibitors were inactive. BN 52021 inhibits the release of the plasminogen activator in vitro and ex vivo (156). Recent studies have shown that, after injection of PAF, plasma protease activity is significantly increased during the first 20 min following the challenge (158).

VI. Pathobiology of PAF

PAF may be involved with a variety of physiopathological conditions, including arterial thrombosis, acute inflammation, endotoxic shock, acute allergic diseases, and early pregnancy.

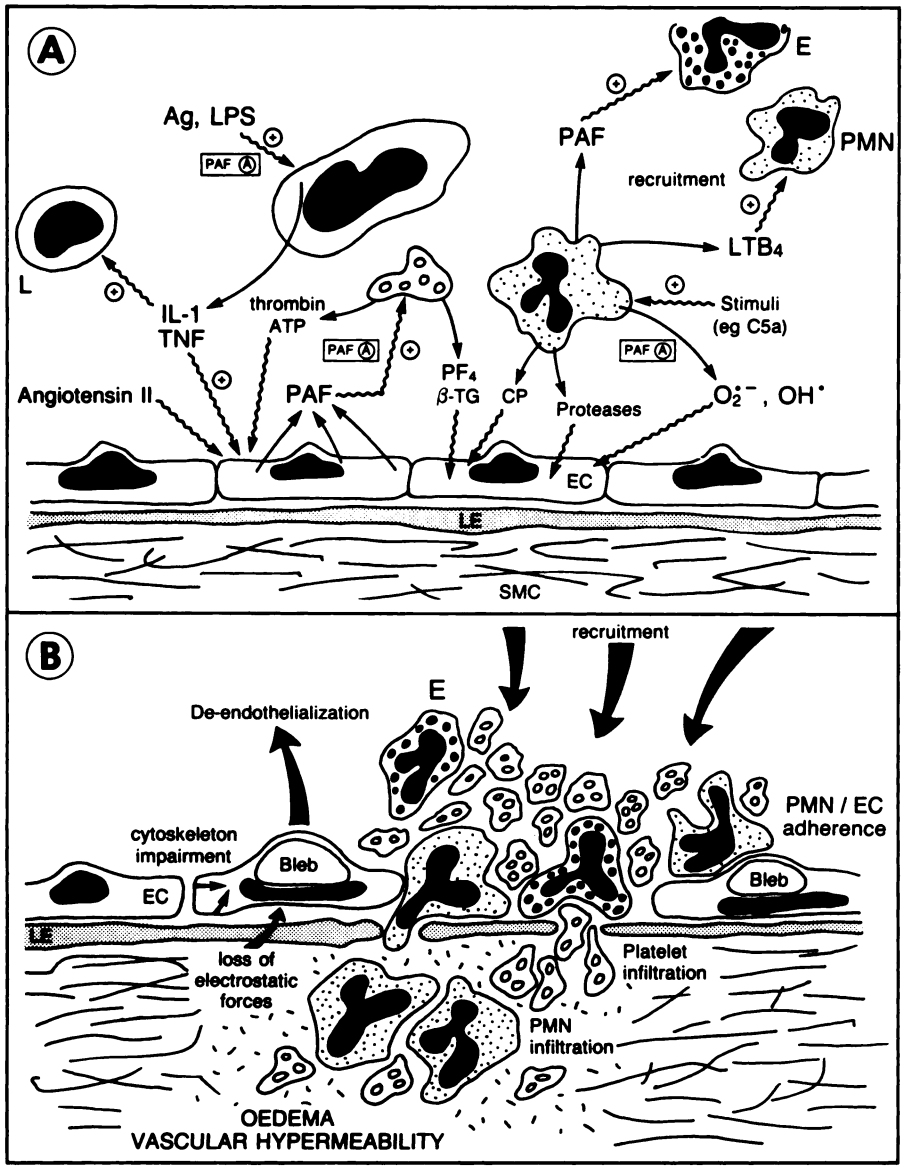
A. PAF and Platelet Participation in Thrombosis

Platelet activation by thrombin is not inhibited by ADP-scavenging agents nor by inhibitors of cyclooxygenase. Accordingly, a so-called "third pathway of platelet aggregation" was postulated as being independent from the cyclooxygenase metabolites and granular ADP (250, 480). Chignard et al. (117, 118) found that a substance identical to PAF is secreted into the supernatants of rabbit platelets stimulated with the Ca^{2+} ionophore A23187, thrombin, or collagen. Like PAF (175, 268), this substance induced an ADP- and AA-independent activation of rabbit (and human) platelets and, accordingly, PAF was suggested to be the actual mediator of platelet activation. PAF is also released by human platelets stimulated with a calcium ionophore or with thrombin (116, 462), particularly when its degradation and subsequent incorporation into the platelet membranes as an acylated derivative are prevented by an acetylhydrolase inhibitor (462).

The hypothesis that PAF mediates the third pathway of aggregation is challenged by recent results (252a, 498), such as those of Adnot et al. (2), showing that three PAF antagonists fail to interfere with thrombin-induced aggregation of aspirin-treated human platelets depleted from their ADP-containing dense bodies by a snake venom component. In accordance, Ro 19-3704 (see below) and other PAF antagonists failed to prevent rabbit platelet deposition in a model expected to demonstrate antithrombotic activity (P. Hadvary and M. Baumgartner, personal communication). *R* (but not *S*) PAF stimulated guanosine triphosphatase (GTPase) activity in platelet membranes dose dependently (71a, 223a, 232). This stimulation is inhibited by BN 52021 and related analogs (71a) and by kadsurenone (232). PAF also stimulates inositol metabolism in platelets (303) and may operate through a guanine nucleotide regulatory protein, distinct from the stimulatory (N_s) or inhibitory (N_i) guanine proteins, which is inhibited by cholera or pertussis toxins (71a, 223a, 232). In contrast, in human neutrophils, the effects of PAF are inhibited by pertussis toxin, suggesting an action on the N_i subunit (262a).

Topical superfusion of PAF onto the exposed guinea pig mesentery causes the formation of a large, dense

and cell margination. In vitro activation leads to enzyme secretion and formation of superoxide and of arachidon- platelet thrombus, invaded and surrounded by leukocytes and spreading over the adjoining vacuolized endothelium (60) (fig. 7, *A* and *B*). Indeed, PAF alters the molecular organisation of cytoskeletal proteins which controls endothelial permeability (81): human endothelial cells stimulated by PAF retract and lose reciprocal contact, while stress fibers disappear or become less regular; such impairments lead to bleb formation. These effects are inhibited by BN 52021, CV 3988, and 48740 RP (81). Fig. 8 summarizes the impairment of endothelial cells by PAF. The etherlipid is a potent amplificator of platelet and leukocyte responses: at very low doses ($10^{-16} \rightarrow 10^{-11}$ M), it dramatically potentiates the release of $O_2^{\cdot-}$ and $OH^{\cdot}$ from PMNs induced by various stimuli (510) and activates platelets to form thrombin, ATP... which, in turn, as IL-1, act on endothelial cells to produce more PAF. Furthermore, platelet factor 4 (PF4) and beta-thromboglobulin (β-TG) possess a well-documented vessel-permeating property (88a). With other cationic proteins released by PMNs, these compounds may neutralize the endothelial polyanions, leading to the loss of selective permeability mediated by electrostatic repulsive forces. Neutrophils and then eosinophils also participate in endothelial cell injury by secreting acid and neutral proteases which destroy basal membrane and activate the formation of C_{5a} anaphylatoxin (fig. 8 for details). Embolization can be prevented or removed with the PLA_2 inhibitor mepacrine, with EDTA, and with the



A : amplification induced by PAF **Ag** : antigen **CP** : cationic proteins **E** : eosinophil
EC : endothelial cells **IL-1** : interleukin-1 **LE** : lama elastica **L** : lymphocytes
LPS : endotoxin **LTB_4** : leukotriene B_4 **PF_4** : platelet factor 4 **β-TG** : beta thromboglobulin
TNF : Tumor necrosis factor

FIG. 8. PAF-induced amplification cycle of endothelial cell injury.

PAF antagonists BN 52021 (60), 48740 RP, and kadsurenone (R. Bourgain, unpublished). The thrombus disruption may be explained by the displacement of PAF by the antagonists which leads to deactivation of fibrinogen binding sites (6, 252).

PAF and epinephrine synergize markedly to induce aggregation of human platelets (175, 268, 485, 486). This may be relevant to the interactions of epinephrine and PAF, released at the same time under different conditions (stroke, different forms of shock). PAF antagonists also suppress aggregation induced by the combination of PAF and epinephrine.

Thus, even though PAF may not be the intracellular mediator of the third pathway of platelet aggregation, its role as a cell-to-cell autacoid deserves investigations. Very recently, it has been shown that PAF antagonists possessing the glycerol backbone (Ro 19-3704 and analogs) also antagonize platelet activation by adrenaline alone, whereas antagonists chemically unrelated to PAF (BN 52021, WEB 2086) are totally inactive (405).

B. Acute Inflammation

PAF increases vascular permeability (13, 75, 320, 360, 370). This effect is accompanied by platelet accumulation, but does not appear to be platelet mediated in the guinea pig (12, 13, 320), rabbit (225, 226), or rat (297, 371), in which the role of neutrophils was also ruled out. The intradermal injection of PAF to the rat is followed by increased vascular permeability, edema, vascular lesion, and thrombosis. An important finding is the presence of platelet thrombi, even though rat platelets are refractory to the direct effect of PAF in vivo and in vitro. In the guinea pig, neutrophils also appear early (around 5 min after the injection of PAF), are marginated and activated within 10 to 30 min, and migrate to extravascular spaces (59, 60). Similar effects were described for the rabbit (225, 226).

Systemic PAF induces hemoconcentration (196, 199, 200, 355), i.e., a generalized increase in vascular permeability, probably due to a direct or indirect (cell-mediated) effect on endothelium (199). Since this is a persistent effect, whereas the accompanying hypotension is reversible and can be renewed when PAF is reinjected, it is clear that permeability disturbance alone does not account for hypotension. Systemic PAF induces an intense ischemia of the rabbit retina, accompanied by a marked plasma leakage and by polyneutrophil sludge, which is neither blocked by platelet depletion nor by reserpine-induced serotonin depletion (75).

PAF induces rat paw edema (132, 182, 484) which is antagonized by dexamethasone, and not by indomethacin or piroxicam (132, 133). The PAF antagonists BN 52021, kadsurenone, and L-652,731 (see table 8) inhibit PAF-induced edema (133, 207, 208, 231, 240, 297, 411, 412). Most interestingly, 48740 RP does not block PAF-induced rat paw edema (297), but antagonizes the accompanying hyperalgesia (S. H. Ferreira, personal communication).

Endogenous production of PAF in close proximity to microvascular endothelial cells appears to be an important step in the development of reverse passive Arthus reaction in rabbits. The edema response is inhibited by intradermal injection of L-652,731 (207) and 48740 RP (208).

Edema induced by the PAF analogs (2-methyl-carbamate and 2-O-methoxy) was antagonized by BN 52021 and by the lipoxygenase inhibitor nordihydroguarietic acid, whereas indomethacin was inactive (133).

The intradermal injections of PAF to humans are said to induce a biphasic inflammatory response, with acute and late-onset components, reminiscent of the response to moderate doses of allergen in sensitized individuals (13). Endothelial swelling and a perivascular infiltrate of mononuclear cells and neutrophils are accompanied by early intravascular accumulation of neutrophils, and late (24 h) appearance of lymphocytes and histiocytes (322). The late component was reported to be inhibited by the antiallergic compound sodium cromoglycate injected together with PAF into the skin at a high concentration (29). These inflammatory effects of PAF, similar to those of vasculitis, may be related to the evidence for the occurrence of basophilic degranulation and release of PAF in systemic lupus erythematosus (96) and to the reported activation of inflammatory cells by immune complexes (95).

Nevertheless, Henocq and Vargaftig (209) failed to identify the late-onset inflammatory response to PAF in humans under conditions when allergens displayed their expected effects. However, they noted that the prominent eosinophilic infiltration that follows the allergen injection into the human skin within 24 h is also present for PAF and, most interestingly, is limited to allergic patients.

C. Asthma and Systemic Anaphylaxis

The involvement of PAF with asthma (reviewed in ref. 482a) is suggested by its ability to induce bronchoconstriction, lung inflammation, and hyperresponsiveness. The first two effects, but not the latter, are shared by other potential mediators of inflammation, such as histamine or AA metabolites. Concrete evidence has accumulated in favour of the involvement of peptidyl leukotrienes in asthma (136), but arguments exist to counter this assumption (488) (reviewed in ref. 482).

There is no perfect animal model for asthma. A systemic active anaphylactic shock in the guinea pig, in fact a histamine-dependent event (143), is frequently used, even though antihistamines are notoriously inactive therapeutically. Models of passive homologous and heterologous systemic anaphylaxis are used and are better controlled by the PAF antagonists BN 52021 (68, 125, 264, 470, 494) (reviewed in refs. 63, 71 and 482a) and WEB 2086 (102, 104, 378a) than active shock (see below). Ro 19-3704 failed to suppress systemic shock, even though it antagonized all tested effects of PAF in the

guinea pig. This may result from the complexity of systemic shock, involving altogether PAF-dependent and -independent mechanisms, since Ro 19-3704 is effective in blocking bronchoconstriction by aerosolized antigen (125).

Effectiveness of some, but not of all, PAF antagonists in passive systemic anaphylaxis cannot thus be used as a definite argument against PAF involvement in shock, but confirms that other mediators, particularly histamine, are involved. In agreement with its proposed role as a mediator of anaphylaxis/allergy, PAF is released in vivo during systemic anaphylaxis in the rabbit (364), possibly from circulating platelets (191, 193), but other sources exist, such as the alveolar lining cells and macrophages (296) (see section V D).

Bronchoconstriction by aerosolized PAF or antigen is reduced by Ro 19-3704 and BN 52021 (125). PAF aerosolization is also followed by partial desensitization to aerosolized antigen, whereas animals desensitized to the antigen retain most of their responsiveness to PAF. These results are consistent with a hypothetic role of PAF as an important mediator for intrapulmonary anaphylaxis in the guinea pig, a possibility reinforced by the fact that lyso-PAF is released from anaphylactic guinea pig lungs challenged with intraarterial antigen (358, 393). PAF itself is formed when the intratracheal route is used (168).

Another possible involvement of PAF with asthma concerns its ability to potentiate bronchoconstriction by other agents (321, 489) and thus account for hyperresponsiveness of asthmatic patients. Indeed, asthma is frequently characterized by nonspecific bronchial hyperreactivity, which may be related to inflammatory events in the lungs (see above). Thus, administered systemically or by inhalation, PAF induces an increased airways reactivity, which may last up to 7 days (135). The time course and magnitude of PAF-induced bronchial hyperreactivity are similar to antigen-induced changes in airways reactivity observed in asthmatics (396).

PAF-induced bronchial hyperreactivity in the guinea pig is inhibited by disodium cromoglycate, ketotifen, hydrocortisone, and methylxanthines and by specific PAF antagonists (BN 52021, L-652,731) but not by indomethacin, histamine H-1 antagonists, or beta-adrenergic agonists (354, 355). The pharmacological sensitivity of PAF-induced bronchial hyperreactivity in the guinea pig thus correlates with clinical effectiveness in asthma.

Furthermore, animals made hyperreactive to exogenous spasmogens by prior treatment with PAF also show a reduction in sensitivity to beta-adrenergic agents. Hyperreactivity may result from a nonspasmogenic effect of PAF involving edema, platelet and eosinophilic infiltration, and epithelial damage (279, 280). Indeed, as shown in fig. 9, 6 to 24 h after the i.v. injection of PAF to guinea pigs, a marked infiltration of activated eosinophils is noted, down to the submucosa, accompanied by epithelium damage and scattered free granules (279). PAF may be a potent amplificator of eosinophil response, since it is highly chemotactic for these cells (453, 502) and induces the release of cationic proteins, such as the major basic protein (MBP), which, in turn, increase bronchial hyperreactivity (fig. 10 for details). Assessment of PAF in antigen-induced airway hyperreactivity has been recently confirmed by the use of BN 52021 in both animals (133b, 467) (fig. 11) and humans (P. Guinot, personal communication). Antagonism of airway hyperreactivity by BN 52021 corroborates the inhibition by the drug of antigen-induced eosinophil infiltration in lung tissue (133b).

Until recently, it was difficult to reconcile the failure of PAF to induce histamine secretion from perfused guinea pig lungs with the ability of BN 52021 (264) and WEB 2086 (378a) to block its secretion upon antigen injection to perfused lungs provided from sensitized animals. This has been clarified, since lungs from sensitized guinea pigs release markedly more thromboxane than control lungs and, most importantly, secrete large amounts of histamine upon challenge with PAF, AA, or LTD_4, whereas control lungs fail to do so (276). Under those conditions, the paradox that PAF antagonists should block histamine release during anaphylaxis, even though PAF does not release histamine from nonsensitized lungs, can be understood, sensitization being responsible by one as yet poorly characterized mechanism for turning PAF into a histamine-releasing substance. This is a very important issue, which should lead to test PAF antagonists on sensitized animals only (276).

Simoes et al. (356, 423) demonstrated that the release of PAF and lyso-PAF by antigen-stimulated alveolar macrophages from untreated asthmatics was more than doubled as compared to theophylline-treated subjects.

Theophylline may inhibit the release of PAF and lyso-PAF by the alveolar macrophages in asthmatic subjects.

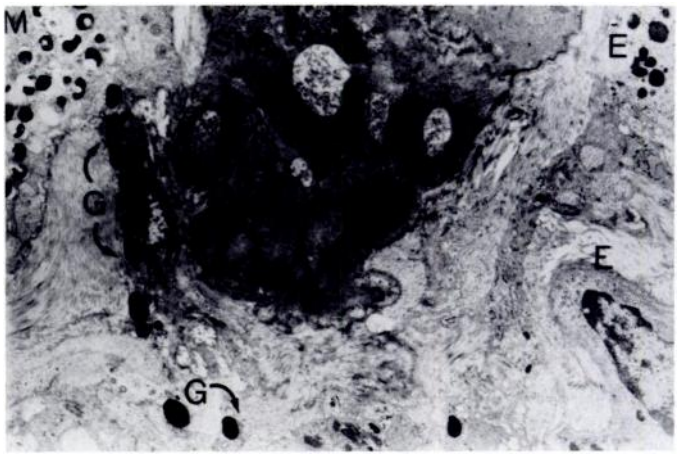


FIG. 9. Electron micrograph of the guinea pig bronchial wall 24 h after the injection i.v. of PAF (100 ng/ml). Tree eosinophil granules (*G*) are seen in the bronchial mucosa, which is edematous. *M*, mast cell; *SM*, smooth muscle; *E*, epithelium. (Figure kindly provided by Dr. A. Lellouch-Tubiana, Hopital Necker Paris.)

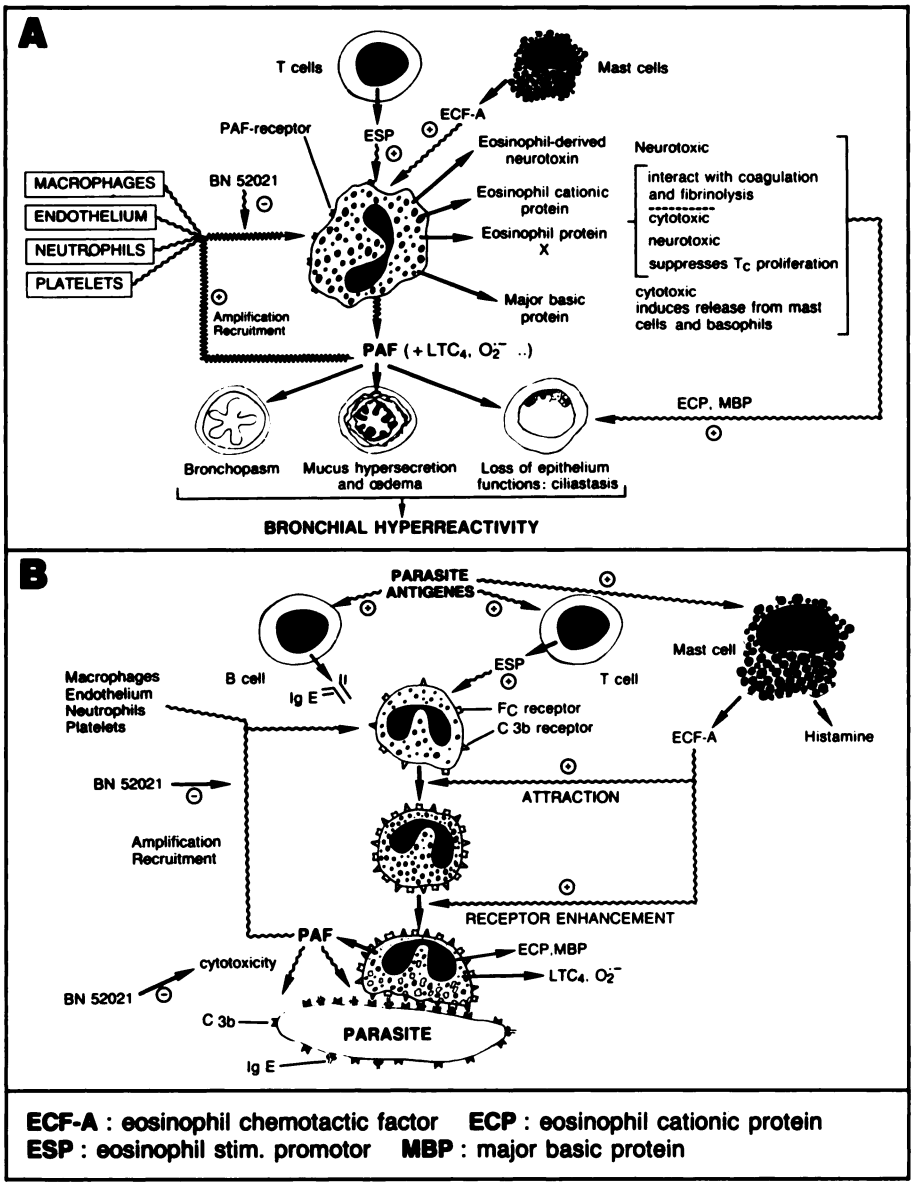


FIG. 10. Hypothetic role of PAF in eosinophil activation: amplification process of bronchial hyperreactivity (*A*) and parasite killing (*B*).

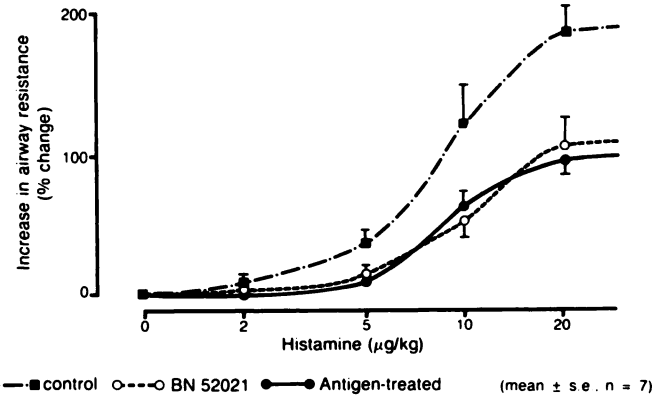


FIG. 11. Effect of BN 52021 on antigen-induced airway hyperreactivity to histamine in guinea pigs (see ref. 467 for details).

Finally, PAF increases mucous output and alters its physical properties (189a, 280, 513). Its topical or systemic administration damages the ciliated respiratory epithelium (189a, 280, 513). Whether these changes are a direct effect of PAF or are secondary to the release of cytoxic substances, such as the major basic protein, by infiltrating eosinophils is not yet known.

D. Transplant Rejection

PAF may be involved in renal transplant rejection since it is released during kidney hyperacute allograft rejection (239). This phenomenon is a humoral-mediated event caused by preformed antibodies involving complement activation, and intravascular platelet aggregation. However, this type of reaction is rarely seen in patients

receiving organ transplants, as most patients experience acute cell-mediated rejection (190a, 402a).

In this case, lymphocyte and monocyte infiltration is readily observed, and platelet involvement is debatable, although there is suggestive evidence, such as accumulation of radiolabeled platelets, in the rejected allograft in humans and animals and the identification of loose platelet aggregates in fine-needle aspirations from human kidney allografts undergoing rejection (190a, 402a). Furthermore, in prolonged rejection, increasing numbers of platelets adhere to the graft vascular endothelial cells, and in irreversible rejection, platelet/endothelial cell aggregates are very prominent. The involvement of platelets in transplant rejection provides the basis for the rationale of trying PAF-antagonists in organ transplantation.

Treatment of rat cardiac allograft recipients with BN 52021 (170–172) alone or in combination with either azathioprine (Az) or cyclosporine (CsA) significantly delayed graft rejection. The combination of Az and PAF antagonist was more effective in prolonging graft survival than the conventional immunosuppressive combination Az plus prednisolone. The combination of BN 52021-L-652,731 or L-653,150 (see table 8) with CsA improved allograft survival to the same extent as prednisolone plus CsA (171) (table 4).

The beneficial effect of PAF antagonists in prolonging graft survival is probably not mediated by a platelet-dependent mechanism, since the PAF antagonists did not antagonize the deposition of radiolabeled platelets in the rat allograft (249). Prednisolone was also inactive against such deposition, whereas a thromboxane antagonist, which also improves graft survival, decreased platelet deposition (249).

An initial step in cell-mediated rejection is accumulation of lymphocytes within the graft. In the rat cardiac transplant model, lymphocyte accumulation occurs during the first 3 to 5 days: BN 52021 had no effect on this accumulation, and interestingly, neither did prednisolone (249). After this period, the increase in lymphocytes in the graft results mainly from proliferation in situ. In cell-mediated rejection, the lymphocyte requires two signals from the macrophage in order to proliferate, foreign antigen presentation and IL-1. Once stimulated, the lymphocytes produce interleukin-2 (IL-2) which is needed for clonal proliferation of T-helper cells and cytotoxic T-cells. As macrophages are stimulated by PAF to synthesize IL-1, the PAF antagonists may have an effect on lymphocyte proliferation (see section VI L for details).

TABLE 4

Effects of PAF antagonists on rat cardiac graft survival. Hearts from Lewis and Brown-Norway F_1 hybrids were transplanted onto the abdominal vessels of rats of the Lewis strain. Rejection was defined as cessation of heart beats determined daily by abdominal palpation by an independent investigator. The rats were divided into five groups. Group I received daily saline injections i.p. Group II received azathioprine (5 mg/kg/day i.p.) Group III received azathioprine as above and the PAF antagonists in two divided doses. Group IV received cyclosporine alone (0.5 mg/kg/day, i.m.). Group V received cyclosporine as above and the PAF antagonists. (Courtesy of Dr. M. Foegh, Georgetown University Hospital, Division of Transplantation, Washington, D.C.)

Drug	Dose (mg/kg/day)	Graft survival (days)
Azathioprine (Az)	5 (i.p.)	10.7 ± 0.6*
Az + prednisolone	5 ± 2	12.8 ± 0.4
Az + BN 52021	5 + 10 (i.m.)	14.8 ± 0.6
Az + L-652,731	5 + 10 (p.o.)	11.4 ± 1.6
Az + L-653,150	5 + 10 (i.m.)	15.3 ± 1.4
Cyclosporine A (Cs)	0.5 (i.m.)	10.3 ± 0.4
Cs + prednisolone	0.5 + 2	18.0 ± 2.9
Cs + BN 52021	0.5 + 10 (i.m.)	14.6 ± 0.5
Cs + L-652,731	0.5 + 10 (p.o.)	15.4 ± 2.2

* Mean ± SE.

E. Cardiac Anaphylaxis

Coronary vasoconstriction induced by antigen in isolated perfused hearts was ascribed to the release of thromboxane A_2 (TxA_2) and leukotriene C_4 (LTC_4). PAF induces a dysfunction resembling that observed in cardiac anaphylaxis (23, 33, 253, 282, 283, 366, 436) and is also released by antigen-challenged hearts (282). The PAF-induced decrease in myocardial contractility and coronary flow in the perfused guinea pig heart are antagonized by BN 52021 and BN 52020 (23, 253, 366, 436), kadsurenone, L-652,731, and L-653,150 (283). BN 52021 considerably blunted the decrease in contractile force and the rise of perfusion pressure due to antigen challenge of perfused hearts from guinea pigs passively sensitized (253, 522). In the hearts from actively sensitized animals, BN 52021 also inhibited the antigen-induced increase in coronary perfusion pressure, this protection being accompanied by a decrease in the release of histamine and thromboxane B_2 (TxB_2) (42).

This decrease in mediator release may vary according to the experimental conditions: protection by PAF antagonists has been reported under conditions where neither the resting nor the antigen-induced increase in release of mediators was affected by BN 52021 and L-652,731 (366, 436) and SRI 63-441 (385b).

F. Kidney Physiology and Immune Disorders

PAF induces the release of thromboxane and prostaglandins from primary cultures of human (14) or rat (407–409) glomerular mesangial cells and from isolated rabbit and/or rat kidneys. In general, this release is inhibited by BN 52021 (331, 332) or kadsurenone (332, 441). This effect is associated with a shape change of mesangial cells which is similar to that observed with angiotensin II and is consistent with contraction of the cells (14). PAF also dose dependently stimulates the formation of reactive oxygen species from cultured mesangial cells (413) and causes a decrease in the planar surface of the glomeruli (138). These effects are inhibited by BN 52021 (138).

Kidneys possess the enzymatic machinery for PAF metabolism: the precursor 1-alkyl-2-acyl-GPC (105);

phospholipase A_2 (369); acetyltransferase (369); and acetylhydrolase (56). Isolated rabbit slices produce a PAF-like material when incubated at alkaline pH (91). Pirotzky et al. (367, 368) showed that PAF is released by isolated perfused rat kidneys and glomeruli, as well as by suspensions of medullary cells but not by tubules upon stimulation by the calcium ionophore A23187 or antigen from immunized rats.

Schlondorff et al. (407–409) characterized the mesangial cells as the major source of PAF in the glomerulus. The possibility that the kidney may generate PAF under normal physiological conditions has been raised in view of PAF bioactivity in human urine (402).

Systemic PAF infusion is associated with profound reduction of renal blood flow, glomerular filtration, and urinary sodium excretion, possibly due the fall of systemic blood pressure (43, 44). PAF also exerts a direct effect on kidney functions (205, 373, 410), since indomethacin protects against the reduction in systemic blood pressure but does not antagonize renal plasma flow and glomerular filtration fall nor urinary sodium excretion (205, 373, 406).

The mechanisms of the differential effects of PAF on systemic and renal microcirculation are not clear, and a local role for vasoconstrictor prostaglandins and/or peptides, such as angiotensin II, was suggested (205, 373).

The role of other metabolites of arachidonic acid, such as the leukotrienes, should be considered since kidneys possess the enzymes to produce leukotrienes, and recent experiments indicate that intrarenal infusion of leukotriene D_4 (LTD_4) results in a reduction of natriuresis, followed by a rebound phenomenon (372), similar to that observed with PAF. BN 52021 inhibits all the physiological effects of the ether lipid on the kidney, suggesting a receptor-mediated action for PAF (205, 373).

Evidence for PAF involvement in renal immune injury has been provided by the observations that PAF is released during kidney hyperacute allograft rejection (239). It has been proposed that PAF participates in glomerular immune complex deposition of experimental serum sickness (98, 397) and in systemic lupus erythematosus (96). In models of nephrotoxic serum nephritis in rats and rabbits (286), it has been reported that PAF antagonists reduce proteinuria and decrease the histopathological lesions. Furthermore, PAF bioactivity could be detected in short-term culture (20 h) of glomeruli isolated from nephritic rats but not in those isolated from control rats (224). These studies cannot differentiate whether the PAF bioactivity was of glomerular origin or was produced by the infiltrating inflammatory cells. Nevertheless, they provide evidence that PAF could be locally generated during glomerulonephritis. A potential role for PAF in renal immune injury and proteinuria is also supported by results obtained with short-duration intrarenal PAF administration in rabbits (97). In these experiments the following changes were observed: (*a*) accumulation of platelets and neutrophils with aggregation and degranulation in glomerular capillaries; (*b*) loss of glomerular fixed negative charges due to binding of cationic proteins released from platelets and neutrophils; and (*c*) transient (hours) and mild proteinuria. The proteinuria could result from a combination of events (i.e., loss of anionic charges and perhaps also a direct PAF-induced increase in capillary permeability) (97, 317). A similar mechanism is certainly involved in Adriamycin-induced kidney impairments, since BN 52021, alprazolam, and triazolam inhibit both proteinuria and the ultrastructural glomerular alterations induced by the drug (153).

PAF has also been postulated to contribute to the pathophysiology of experimental unilateral ureteral ligation (505, 506). Perfusion of isolated hydronephrotic kidneys 72 h after unilateral ureteral ligation with PAF resulted in dose-dependent release of prostaglandins and thromboxane. This PAF effect was inhibited by kadsurenone or CV 3988.

Finally, a recent study has shown that PAF pretreatment in anesthetized dogs practically abolishes natriuresis and diuresis induced by the synthetic [Ser 99-Tyr 126] atrial natriuretic factor (ANF) (458, 459). In contrast, the PAF antagonist BN 52021, which is devoid of effects per se on diuresis and electrolyte excretion, potentiates the effects of ANF (458, 459) and captopril (P. Braquet and J. Baranes, unpublished). Whether this effect is related or not to PAF inhibition is not yet clear.

G. Endotoxic and IgG-induced Shocks

Shock is characterized by systemic hypotension, pulmonary hypertension, endothelial dysjunction (stretched pore phenomenon), and stimulation of different plasma systems (kallikrein, fibrinolysis, clotting) and circulating cells (erythrocyte sludge, stimulation of leukocytes and platelets). It is difficult to admit that this very complex event can be accounted for by a single primary mediator but, based on in vivo pharmacological observations, such a role was ascribed to the arachidonate metabolites, essentially in the rat (131, 346). Studies on rabbits (78) and sheep (420) demonstrated the involvement of mediators other than icosanoids and, accordingly, only a marginal improvement was ensured by aspirin (169). The analogies between endotoxic shock and the effects of systemic PAF in the dog and rat (reviewed in ref. 72) were initially noted by Bessin et al. (43). Terashita et al. (435) then demonstrated that the PAF antagonist CV 3988 improved survival and prevented or corrected endotoxic shock hypotension in the rat. Inarrea (237) showed that shock triggered by i.p. injections of endotoxin in the rat is accompanied by the appearance of PAF in the peritoneal and spleen cells of the intoxicated animals. Further evidence was provided by Doebber et al. (149), who demonstrated that the PAF antagonist kadsurenone prevents PAF and endotoxin-induced hypotension in the rat to a similar extent, and that animals made tolerant to endotoxin still respond to subsequent PAF injections. Since then, a large number of chemically

unrelated PAF antagonists including L-652,731 (418), BN 52021 (3, 4, 158, 159), SRI 63-072 (479), WEB 2086 (102), SRI 63-441 (198), FR-900452 (343a), and Ono 6240 (318) have been shown to protect against shock in animals.

Furthermore, a recent study of Feuerstein et al. (163) demonstrates that PAF may also be involved in trichothecine-induced intoxication in the rat by using BN 52021.

A possible site of action of PAF and endotoxin is the bowel, since it was demonstrated that necrotizing bowel lesions can be obtained in the rat by the combination of endotoxin and PAF, under conditions where either alone is inactive (186, 224a) (see also section VI H).

The involvement of PAF in endotoxemia has been recently confirmed by Touvay et al. (468) using isolated guinea pig lung parenchymal strips from both normal and endotoxin-treated animals. In the latter, a significant desensitization to PAF in comparison to the control group was seen, the autacoid being unable to contract the strips from intoxicated animals. Endotoxin aerosol in guinea pigs induces platelet accumulation in the lung which is reduced by both BN 52021 and WEB 2086 (30). PAF, as endotoxin, induces hyperglycemia (347, 471) which is prevented by BN 52021 (A. Etienne and P. Braquet, in preparation).

As stated above, endotoxic shock is a very complex event, and there are notable species differences among various experimental models. Since PAF may have different receptors, on the same or on different cell types, it is not surprising that a single autacoid antagonist should fail to prevent all of its effects (reviewed in ref. 72). The as yet vague desensitization of adrenergic receptors by endotoxin (144, 332a) or PAF (5, 66, 133c), possibly related to a phosphorylation process (421a), may be the key for better control of endotoxic and septic shock in humans.

PAF antagonists also protect against IgG-induced shock in the rat. BN 52021 (162, 400), BN 52020, and BN 52022 (401); kadsurenone; L-652-731 and L-653,150 (148, 418); and SRI 63-072 (479) prevent and reverse hypotension and the lysosomal enzyme release induced by the immune challenge. Similar protection has also been observed in immune experimental pancreatitis (S. Jancar and P. Sirois, in preparation). In contrast, CV 3988 is ineffective in attenuating acute hemorrhagic hypotension, suggesting that PAF is not involved in this type of shock (460a).

H. Gastrointestinal Ulceration

PAF is the most potent ulcerogen yet described in the rat (186, 391). This effect is not mediated via platelets or cyclooxygenase products, nor via histamine or adrenergic receptors (391). PAF-induced ulcerations mimic the gastrointestinal impairments obtained after endotoxin administration (67, 391, 499): the damage to the gastrointestinal tract in both models was characterized by vascular congestion extending throughout the mucosa and sometimes to the submucosa. As endotoxin, PAF also induces hemorrhagic damages in the stomach and small intestine (186), but not in the distal colon. The reason for this resistance of the distal colon to these damaging agents is not yet clear (67, 499).

The strongest evidence for a role for PAF in endotoxin-induced gastrointestinal necrosis observation was that, at doses which inhibit PAF-induced gastrointestinal damage, BN 52021 (67, 449), CV 3988 (499, 500), and Ro 19-3704 (499) inhibit endotoxin-induced gastrointestinal ulcerations (fig. 12). In confirmation, Lagente et al. (263) showed that PAF release from jejunum of endotoxin-treated rats is significantly increased over controls.

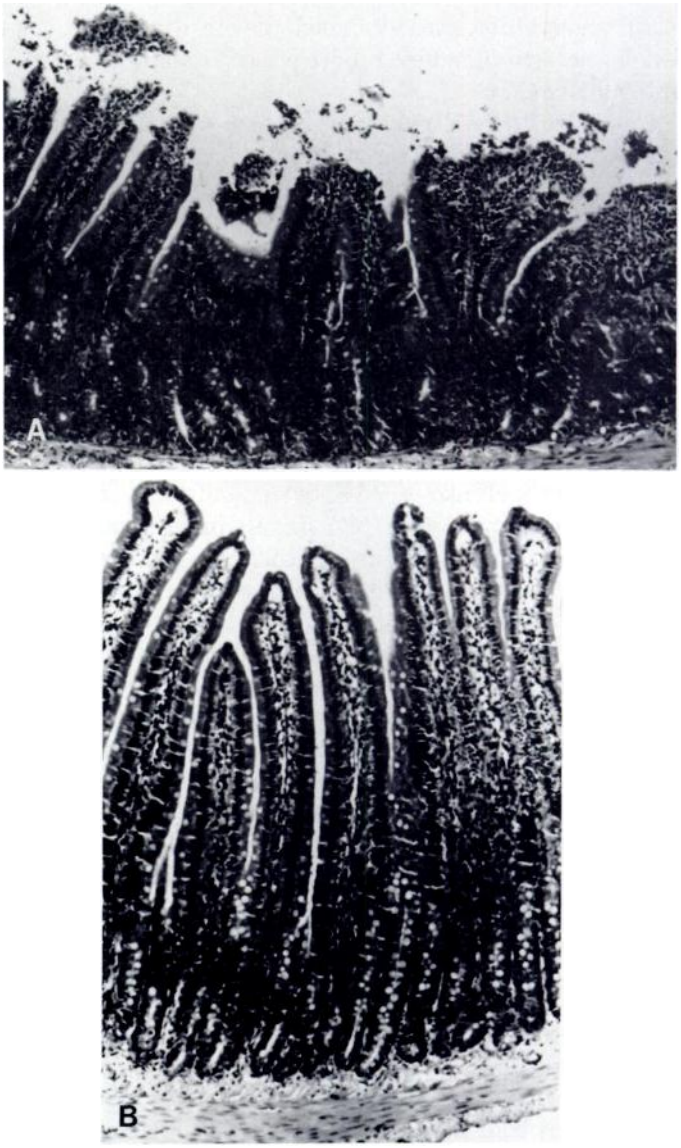


FIG. 12. Light micrographs of 4-μm sections of duodenum of rat 20 min after an i.v. infection of *E. coli* lipopolysaccharide (*A*) (25 mg/kg). The section on the *left* is from a rat pretreated only with vehicle, while that on the *right* was pretreated with BN 52021 (10 mg/kg i.v.). Note that the section on the *left* is characterized by extensive necrosis involving the top half of the villi. Cellular debris is visible in the lumen. Conversely, the section from the rat pretreated with BN 52021 is intact (*B*). There are no necrosis, vasocongestion, or hemorrhage. H&E, × 110. (Courtesy of Dr. J. L. Wallace.)

The involvement of PAF in other types of gastrointestinal ulceration is less apparent: PAF may be involved in necrotizing enterocolitis (224) in gastric mucosal damages and mucosal formation of eicosanoids in rats treated with ethanol. Indeed, BN 52021 (67) and CV 3988 (499) significantly reduced gastric lesions caused by alcohol.

Furthermore, mucosal formation of LTC (361) tended to be reduced in rats treated by BN 52021. However, gastric formation of PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 is not affected by BN 52021 (B. Peskar, personal communication). In contrast, Wallace et al. demonstrated that Ro 19-3704 did not significantly affect the extent of ethanol-induced damage (499).

BN 52021 and triazolam also partially protected against restraint stress-induced gastric damage in the female rat, atropine being more effective than both PAF antagonists (67).

In contrast, PAF may not appear to be involved in gastric hypersecretion, in pylorus-ligated rats, and in aspirin-, phenyl-, or butazone-induced gastric damage, since BN 52021 only afforded a mild or nil protection, under conditions where ranitidine was effective (67). PAF may be involved in postischemic lesions of the gastric (151a) and the intestinal (C. Tagesson and D. Lewis, in preparation) mucosa.

I. Inflammatory and Allergic Skin Diseases

The various effects of PAF, particularly inflammatory responses in human skin following its intradermal injection, suggested that PAF may be involved in the pathogenesis of inflammatory skin disorders, mainly psoriasis and cold urticaria. PAF was identified in the psoriatic scale and in fluid collected in chambers placed over psoriatic lesions (292, 293). The source of PAF found in this fluid is unknown and includes inflammatory cells present in lesional skin and the resident population. Other mediators have also been identified in psoriatic skin, particularly products of the lipoxygenase pathway of arachidonic acid, leukotrienes (61), monohydroxyeicosatetraenoic acids (HETEs) (86), as well as IL-1 (85) and the complement fragment C5a (452). Therefore, although PAF induces LTB_4 and HETEs release by normal keratinocytes (M. Braquet, in preparation), the relative importance of these various mediators in the development and maintenance of the psoriatic lesion remains unknown.

Release of a PAF-like lipid into blood has been demonstrated in cold urticaria patients following cold challenge (187). Whether the PAF-like lipid is released directly as a result of the challenge, or secondarily from cells or tissue stimulated by other mediators such as histamine and prostaglandin D_2 remains unclear. The antidepressant, doxepin, was reported to reduce both the urticarial response and the amount of the PAF-like lipid released following challenge (187), but this was not confirmed (Palma-Carlos, personal communication).

J. Retinal and Corneal Diseases

PAF is produced by embryo chick retina upon stimulation with neurotransmitters such as acetylcholine and dopamine (82). High affinity specific binding sites for PAF are found in rat isolated retina (M. Doly and P. Braquet, in preparation).

Furthermore, retinal circulation is a target for PAF, since it induces intense extravasation and sludge in the rabbit (75): the angiofluorographic pictures obtained mimic the pattern of different retinopathies. In addition, (*R*) [but not (*S*)] PAF and lyso-PAF significantly impair the b wave of the electroretinograph of the rat isolated retina (151), and these effects are dose dependently inhibited by BN 52021 and related antagonists (151). Interestingly, in frog eyecups incubated in a normally permissive medium, BN 52021 (10^{-6} M) increases shedding and phagocytosis of rod outer segments (N. Bazan, personal communication).

PAF may be also involved in corneal diseases, since BN 52021 given topically antagonizes immune keratitis and activates corneal regeneration (491a). Interestingly, BN 52021 also inhibits the transient increase in intraocular pressure induced by laser iridal burns (491a); such a phenomenon is also inhibited by indomethacin and may result from the release of PGE_2 induced by PAF, observed in rabbit iris smooth muscle (521a).

K. Central Nervous System

A potential role for PAF in the central nervous system (CNS) was suggested, by the discovery that triazolobenzodiazepines (alprazolam or triazolam) (255, 256) and some benzodiazepines (107–109) antagonize PAF. PAF or PAF-like phospholipids may be involved in cell-to-cell interactions in mature brain, the antagonism of this process contributing to the therapeutic effects of triazolobenzodiazepines, while in the developing nervous system PAF may play a role in cell differentiation (256). One observation supporting this suggestion is the finding of a neuronal dysfunction in individuals with Zellweger syndrome, a genetic disorder in which there is a lack of enzymes needed for the synthesis of ether-phospholipids (247a). This possibility is supported by the fact that the brain contains relatively high levels of enzymes for PAF metabolism.

Recent studies of Kornecki et al. (256) demonstrate that PAF induced growth arrest and morphological differentiation of the cloned neuronal cell line NG 108-15. This effect is concentration and time dependent (256). Only when the dose of PAF is increased above levels that induce maximal differentiation, cytotoxicity appears. The finding, that PAF is cytotoxic to neural cells, raises the possibility that PAF may be one of the factors responsible for the irreversible neuronal degradation associated with spinal cord injury, trauma, and stroke (256). The biochemical mechanisms underlying the effects of PAF on neurons may be related to a rise in free

intracellular Ca (2, 254). Such effect may be involved in the PAF-induced stimulation of prolactin and growth hormone from rat anterior pituitary tissue (188).

Alprazolam is used as an antidepressant agent, particularly in the treatment of panic disorders. Kornecki and Erlich (256) reported that panic attacks are accompanied by a 5- to 20-fold increase in blood levels of platelet factor 4 and other factors, which mark for platelet activation. The panic attacks and the increased blood levels of the platelet markers were prevented by alprazolam, suggesting that PAF may be involved in the development of panic disorders, and that alprazolam is effective in this condition because of its PAF-antagonist properties.

PAF antagonists may enhance neuronal recovery after brain ischemia, since in anaesthesized dogs with multifocal ischemia, kadsurenone enhanced early postischemic recovery (252b). Similar results were recently obtained with BN 52021 in a related model of brain ischemia in gerbils (B. Spinnewyn and P. Braquet, in preparation). Since PAF antagonists had no effect on platelet accumulation, their beneficial effect on postischemic recovery should be by platelet-independent mechanisms.

L. Immune Response

PAF may regulate the lymphocyte functions either (*a*) indirectly since PAF produces leukotrienes formation in lungs (96), which in turn are potent modulators of lymphocyte functions (388) or (*b*) directly by acting on T-cell and macrophage functions.

When added at 10^{-12} M to adherent rat spleen monocytes stimulated with lipopolysaccharide (LPS), PAF induces a significant increase in interleukin-1 (IL-1) synthesis and release (363); in contrast, at 10^{-7} M PAF, a decrease in both IL-1 synthesis and release was observed (363a). BN 52021 and related antagonists significantly reverse these effects (363a). Such PAF-induced IL-1 synthesis and release may explain the antitumour activity of 1-O-alkyllysophospholipids (ALPs) (38, 137, 155) (see section VI N) whose effects may partly be mediated by the generation of highly tumouricidal immune-competent cells from the monocyte-macrophage lineage (38, 328), which are able themselves to produce a large amount of PAF upon stimulation (8, 15, 89, 90, 314, 316). A positive feedback between IL-1 and PAF may be thus involved in the amplification of the immune response. PAF and PAF antagonists also modulate synthesis, release, and effects of interleukin 2 (IL-2), but their effects appear complex and not well understood. When human lymphocytes are cultured in the presence of the mitogen phytohemagglutinin (PHA), their proliferation is markedly impaired by (*R*)-PAF ($10^{-8} \rightarrow 10^{-6}$ M) (389). PHA-dependent lymphocyte proliferation is dependent upon IL-2 production which is decreased significantly by (*R*)-PAF ($10^{-8} \rightarrow 10^{-6}$ M). A similar but lower suppression is observed with ethoxy-PAF. Lyso-PAF and (*S*)-PAF are inactive, suggesting a receptor-mediated mechanism (363).

Several cyclooxygenase metabolites of arachidonic acid, in particular prostaglandins of the E series, can exert a powerful suppressive effect on lymphocyte proliferation. Indeed, while indomethacin has no significant effect per se, its concomitant use in the proliferation assay completely prevented the PAF-induced suppression, indicating that PAF may exert its effect through some cyclooxygenase metabolites, e.g., PGE_2 (363, 363a).

BN 52021 and the related compound BN 52020 at micromolar concentrations completely reversed the inhibition caused by (*R*)-PAF or ethoxy-PAF (363), but also showed some suppressor cell-inducing properties of their own (30%) (390). Interestingly and in contrast, when PAF and the two nonhydrolyzable PAF agonists PR 1501 and 1502 (501) are added to a human T-lymphoblast preparation in the presence of exogenous IL-2, Ward et al. (501) observed enhanced proliferation. In these conditions, PAF antagonists as well as the PAF synthesis inhibitor L-648,611 (385a) are effective inhibitors of IL-2-induced proliferation, as measured by [^{3}H] thymidine incorporation (28, 501). Therefore, the effects of (*R*)-PAF and PAF antagonists appear reversed and depend on the presence or absence of exogenous IL-2.

Until recently, it was thought that, unlike other leukocytes, lymphocytes were not able to produce PAF (87a), but recent findings did not confirm this assumption. (*a*) Lymphocytes are able to produce lyso-PAF upon stimulation with the calcium ionophore A23187 (243). (*b*) Large granular lymphocytes secrete PAF after F_c receptor stimulation (291a). (*c*) Human leukemic cells of B and T origin are able to release PAF-like material after appropriate stimulation with A23187, PHA, and acetyl-CoA (81a). IL-2 may be an appropriate stimulant of PAF release, the lymphokine being required for activation or synthesis of acetyltransferase which may be mediated by protein phosphorylation (501).

M. Pregnancy and Ovoimplantation

PAF may be the first physiological signal produced by the embryo for maternal recognition of pregnancy. It is present in human amniotic fluid (47, 48, 335), and the enzymes involved in PAF metabolism are found in human amnion tissue (22, 47) where PAF is synthesized (47). PAF is also present in the rabbit uterus during early pregnancy in concentrations well above those required to initiate cutaneous permeability (12).

The mammalian embryo produces PAF within 6 h of fertilization at levels which cause significant intravascular platelet activation (348). In the mouse (349, 350), marmoset (352), and human (350), this embryo-derived PAF induces a mild thrombocytopenia during the first days of pregnancy. In vitro production of PAF decays with time in culture (351) which may result from the culture process, since freshly collected embryos still produce PAF. This reduction in PAF production may ex-

plain in part the decreased capacity of cultured embryos to result in pregnancy, since a correlation between inability of cultured embryos to produce PAF and to result in pregnancy was found (352). These observations suggest that PAF production is a prerequisite for pregnancy, and that it could be used as a marker for embryo viability.

In mammals, the success of implantation requires a rapid metabolic burst (blastocyst activation). Embryo-derived PAF may promote platelet activation and subsequent release of factors which stimulate the blastocyst activation and consequent implantation (352). Indeed, blastocyst activation is promoted by platelet-dependent serum factors. Much attention has centered on an "early pregnancy factor" (EPF) which can be detected in the serum of mice, sheep, and humans within the first 24 h of fertilization. In mice, it was detected 6 h after mating, i.e., before the fusion of the male and female pronuclei.

Recent work suggests that embryo-derived PAF may be this "ovum factor" (352). Indeed, synthetic PAF induces the expression of EPF within an hour of injection in mature female mice at all stages of the oestrus cycle except for metaoestrus. Furthermore, culture fluid from fertilized ova has been shown to induce platelet activation and thrombocytopenia, the active agent in the culture fluid being identified as PAF (349, 350).

Interestingly, Braquet et al. (65) identified in human and mammalian urines from pregnant subjects a series of moderately active PAF antagonists [endogenous PAF-inhibiting factors (EPIFs); see section VII B 2 b ii]. EPIFs may constitute a negative feedback to the necessary increase in PAF production by the embryo during pregnancy.

As the lipoxygenase inhibitor EP10045 or indomethacin, BN 52021 markedly reduces ovoimplantation when given in the lumen of uterine horn (1). In contrast, only moderate effects were recorded when the PAF antagonist was given p.o. (1). Confirmation of the role of PAF as an important mediator in the establishment of pregnancy may lead to improvements in in vitro fertilization and open up the possibility that PAF antagonists may be used as local contraceptive agents.

N. PAF-related Phospholipids in Cancer Chemotherapy

Phospholipid analogs of PAF, the ALP, may provide a new approach to cancer chemotherapy (38, 137, 155). These ether lipids possess an unusually broad range of biological activities, macrophage activation, malignant cell differentiation, and direct cytotoxicity, all thought to be membrane mediated. Unlike most antitumour agents, these analogs do not appear to have a direct effect on DNA synthesis or function and are nonmutagenic (39). The methoxy analog of PAF, one of the most potent ALPs, is strongly associated with surface and intracellular membranes (432a). This product inhibits the uptake of essential nutrients (choline, palmitic) by the HL-60 cells (432a). ALPs may also inhibit sialyl transferase (18a), the phospholipid-sensitive Ca^{2+}-dependent protein phosphorylation system (206a). In addition, ALPs may disturb membrane organization, most tumour cells being deficient in 1-O-alkyl cleavage enzymes (432b). The antitumour activity of ALPs may partly be mediated by the generation of highly tumouricidal immune-competent cells from the monocyte-macrophage lineage (38, 328). The process of macrophage activation is still unclear (328), but an activation of IL-1 by ALPs is possible (363a). Interestingly, the uptake of PAF or its methoxy analog is not inhibited by PAF antagonists, BN 52021 and kadsurenone (381a), suggesting that the uptake of ether lipids is not mediated by PAF receptor in HL-60 cells. Application of ALPs p.o. is possible due to effective resorption.

A number of derivatives of ALP have been synthesised, such as 1-hexadecylthio-2-methoxymethyl-*rac*-glycero-3-phosphocholine (BM-14-440) (213), SRI 62-834 and analogs (223), the compound 1-octadecyl-2-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH_3) (442), and various *rac*-(2-alkoxyalkyl)phosphocholines (58). Clinical phase 1/II trials and pharmacokinetic studies with BM-14-440 p.o. have recently been initiated (213).

The most potent ALPs with selective antitumour activity are those with acetamide or methoxy substituents at the *sn*-2 position of ALP (220, 220a, 220b). As PAF and related agonists are poor antitumour agents, an inverse relationship appears to exist between alkyl ether phospholipids exhibiting PAF activity (hypotensive, inflammatory, and allergic reactions) and those possessing selective antitumour properties.

A recent meeting had reviewed different aspects of this new field of research (508).

O. Experimental Cirrhosis

Experimental cirrhosis with carbon tetrachloride is accompanied by a hyperdynamic circulatory state with an increased cardiac output and reduced arterial pressure and peripheral resistances. Plasma PAF is significantly elevated in this model (100). BN 52021 antagonized the hemodynamic effects induced of carbon tetrachloride and normalized the peripheral resistance and cardiac ejection volume (495).

VII. Regulation of Function and Metabolism of PAF

A. Inhibition of PAF Biosynthesis and Metabolism

Glycerophosphocholine (GPC), including the membrane-bound alkylacyl-GPC precursor of PAF, can be synthesized by the transmethylation of corresponding glycerophosphatidylethanolamine (GPE) (see section III C 2). Two inhibitors of phospholipid methylation, 3-deazaadenosine and L-homocysteine, reduce the synthesis of PAF by thrombin-stimulated rabbit platelets, through mechanisms other than inhibition of phospholipid methylation (461).

Two synthetic phospholipid analogs, 2- and 3-(N-palmitoylamino)propyl phosphorylcholine, were found to

inhibit the lyso-PAF-acetyl transferase of rat spleen microsomes with an IC_{50} of 5 μM. They also inhibit A23187-induced biosynthesis of PAF by mouse peritoneal macrophages at 25 to 50 μM (385a). However, their enzymatic specificity remains to be established.

At concentrations above those required for receptor blockade, two PAF antagonists were found to interfere with the metabolism of PAF in vitro. BN 52021 inhibits the conversion of PAF by intact platelets at 10 μM. This effect was ascribed to the inhibition of transmembrane movements of PAF, since BN 52021 had no effect on PAF metabolism by lysed platelets (262). In another study, the PAF-receptor antagonist, L-652,731, inhibits the deacetylation of PAF by intact platelets with an IC_{50} of 50 μM and maximal effect of 70% inhibition at 100 μM. At this concentration, it inhibits the reacylation of lyso-PAF by 20% only by both intact and homogenated platelets (L. Touqui, in preparation).

These data correlate with inhibition by BN 52021 (262) and related ginkgolides (266) of conversion of PAF into 1-O-alkyl-2(*R*)-acylglycero-3-phosphocholine and phosphatidic acid synthesis (424) by intact rabbit platelets without inhibition of acetyltransferase.

B. Antagonists of PAF

1. Nonspecific inhibition of the effects of PAF. Drugs which interfere with intracellular calcium also interfere with the cell response to PAF in vitro and in vivo. These include agents which act directly, such as calcium channel antagonists (127, 248, 305, 415), calmodulin inhibitors (202, 242, 284, 285), calcium chelators (248), and local anaesthetics (111), or indirectly by modulating the level of cyclic nucleotides, e.g., prostaglandin I_2 (PGI_2) or PGE_1 (80, 94, 99), and β_2-agonists, such as salbutamol (23) for cAMP, the molsidomine metabolite, Sin-1, for cyclic guanosine monophosphate (cGMP) (114), and phosphodiesterase inhibitors (313, 486).

Other compounds, such as cromoglycate (29), thyrotropin-releasing factor (TRH), and its analog, MK 771 (164, 165), naloxone (165), ticlopidine (269), atropine (425), some quaternarized derivatives of promethazine (134), and C-reactive protein (493), can counteract the effects of PAF. A similar nonspecific inhibition was observed with inhibitors of phospholipase (60) and antagonists of thromboxane and leukotrienes (123, 317, 429).

The antidepressant agent doxepin was reported to alleviate clinical manifestations and the release of PAF-like lipid in patients suffering from cold urticaria; no inhibition of histamine or neutrophilic chemotactic activity release was recorded in the same trial (187).

The in vivo anaphylactic-like effects of PAF in the guinea pig are not inhibited by anti-H_1 or anti-H_2 agents, except when they are combined with aspirin, indomethacin, or salicylic acid, which are inactive alone (491). In contrast, sulphinpyrazone at very high doses blocks PAF-induced bronchoconstriction and platelet secretion (119).

2. Specific inhibition of PAF. Specific PAF antagonists are useful tools for defining the biological roles of PAF and conformational properties of PAF receptor sites (reviewed in refs. 64, 69, 70, and 488). They can be conveniently classified into four different groups as follows.

a. PAF-RELATED ANTAGONISTS. These compounds have been obtained by three different chemical approaches.

i. Nonconstrained backbone. In this series, the antagonists derive directly from the PAF framework. The first compound described in this family is CV 3988 (fig. 13, 1) (Takeda) which incorporates an octadecyl carbamate in position 1, methylether in position 2, and thiazolium ethyl phosphate in position 3 (457). CV 3988 is an orally active and potent antagonist: the K_i values of CV 3988 for the specific binding of [^{3}H]PAF to human, rabbit, and guinea pig platelets are, respectively, 1.6×10^{-7} M, 1.2×10^{-7} M, and 1.8×10^{-7} M. A weak agonistic activity was found only at high concentrations. The first reports by Terashita et al. (457) claimed that CV 3988 specifically inhibited PAF-induced platelet aggregation. However, recent studies have shown some antagonistic effect against AA and ADP (337) and collagen and A23187 (133a) at high CV 3988 concentrations. The inhibition of PAF-induced platelet aggregation by CV 3988 is accompanied by an inhibition of the membrane production of phosphoinositides (421). CV 3988 inhibits PAF-induced hypotension, thrombopenia, and hemoconcentration, and antagonizes endotoxin shock in the rat (455). CV 3988 also counteracts the decrease in blood pressure induced by unclipping of the renal artery in the one-kidney one-clip hypertensive rat (302).

A new analog CV 6209 (fig. 13, 3) has a N-acetyl carbamyl methyl pyridinium side-chain in place of the phosphorylcholine group. It is about 80 times more potent than CV 3988 in vitro and in vivo, but is poorly absorbed p.o. (456).

Replacement of the phosphoryl ethyl thiazolium moiety of CV 3988 with a heptamethylene thiazolium on C_3 yielded another group of antagonists among which Ono 6240 (fig. 13, 7) is the most potent one (318). Ono 6240 inhibits PAF-induced guinea pig platelet aggregation (IC_{50} = 0.2 μM) and hypotension and bronchoconstriction in rats and guinea pigs at 0.1 to 1 mg/kg i.v.

A similar approach was followed by Hoffmann La Roche's group, leading to potent antagonists, such as Ro 19-3704 (fig. 13, 4, the most efficient), Ro 19-1400, Ro 18-8736, and Ro 18-7953 (25, 79). The IC_{50} against PAF (4 nM)-induced rabbit platelet aggregation of these antagonists ranges between 10^{-8} and 10^{-6} M. Ro 19-3704 inhibits PAF-induced aggregation of rabbit, human, and guinea pig platelets, as well as bronchoconstriction, hypotension, thrombocytopenia, leukopenia, and vasopermeation in the guinea pig. Furthermore, it prevents PAF-induced activation of alveolar macrophages and the formation of thromboxane by PAF-stimulated isolated perfused guinea pig lungs. Ro 19-3704 fails to counteract bronchoconstriction of homologous passive systemic an-

aphylaxis but antagonizes bronchospasm due to aerosolized antigen (125).

Related antagonists have been recently described by Sandoz (197, 200, 479, 511, 512): SRI 63-119 and SRI 63-072 (fig. 13, 5, 6). These compounds, which did not exhibit enantiospecificity, inhibit PAF-induced human platelet aggregation with $IC_{50} = 3.8 \times 10^{-6}$ M and 2.23×10^{-5} M, respectively. SRI 63-072 and SRI 63-119 inhibit PAF-induced hypotension in the rat and in the guinea pig, and they counteract PAF-induced bronchospasm in the latter species. Other related compounds with moderate activity, such as SaRI-62586 and SaRI-62436, have been described (271).

A novel amidophosphonate analog has been recently described as a potent inhibitor of aggregation induced by PAF (433).

Finally, potent antagonists were obtained by replacing the phosphoryl group by an ester linkage [i.e., RU 45703 (fig. 13, 2); IC_{50} (rabbit PRP aggregation): 8×10^{-6} M] (509).

Taken together, structure-activity relationships for the carbons of glyceryl framework of nonconstrained PAF-related antagonists are the following (antagonistic potency): C_1, —O—CONH— > —NHCO—O— > —O—CO— > —O—; C_2, —O—COOMe > —O—CONHMe ≥ —O—COMe > OMe; C_3, —O—CO— > —O—CO—O > —NHCO— > —$NHSO_2$— > —O—PO_2— ~ —PO_2—O > O—PO_2—O.

ii. Constrained backbone. Moderately active PAF antagonists were produced from cyclization of the PAF framework, such as the Sandoz piperidine-derived SRI 63-073 (272) (fig. 13, 9) or the dioxanone-related Hoffmann-La Roche series (79) (fig. 13, 8). SRI 63-073 synthesis was designed by combination of thiamine phosphate (which displays a modest PAF-inhibitory effect in vivo) with a modified PAF framework. The potency of these compounds is relatively low: SRI 63-073 inhibits PAF-induced human and guinea pig platelet aggregation with $IC_{50} = 3.77 \times 10^{-5}$ M and 1.5×10^{-5} M, respectively, and PAF binding with $IC_{50} = 3.4 \times 10^{-6}$ M.

iii. Tetrahydrofuran derivatives. Sandoz has also synthesised a series of tetrahydrofuran-related PAF antagonists related to the PAF framework (241). The most potent one, SRI 63-441 (fig. 13, 10), is a specific inhibitor of PAF-induced human platelet aggregation, with an IC_{50} of 3.3 μM. SRI 63-441, at 1 mg/kg i.v., completely pro-

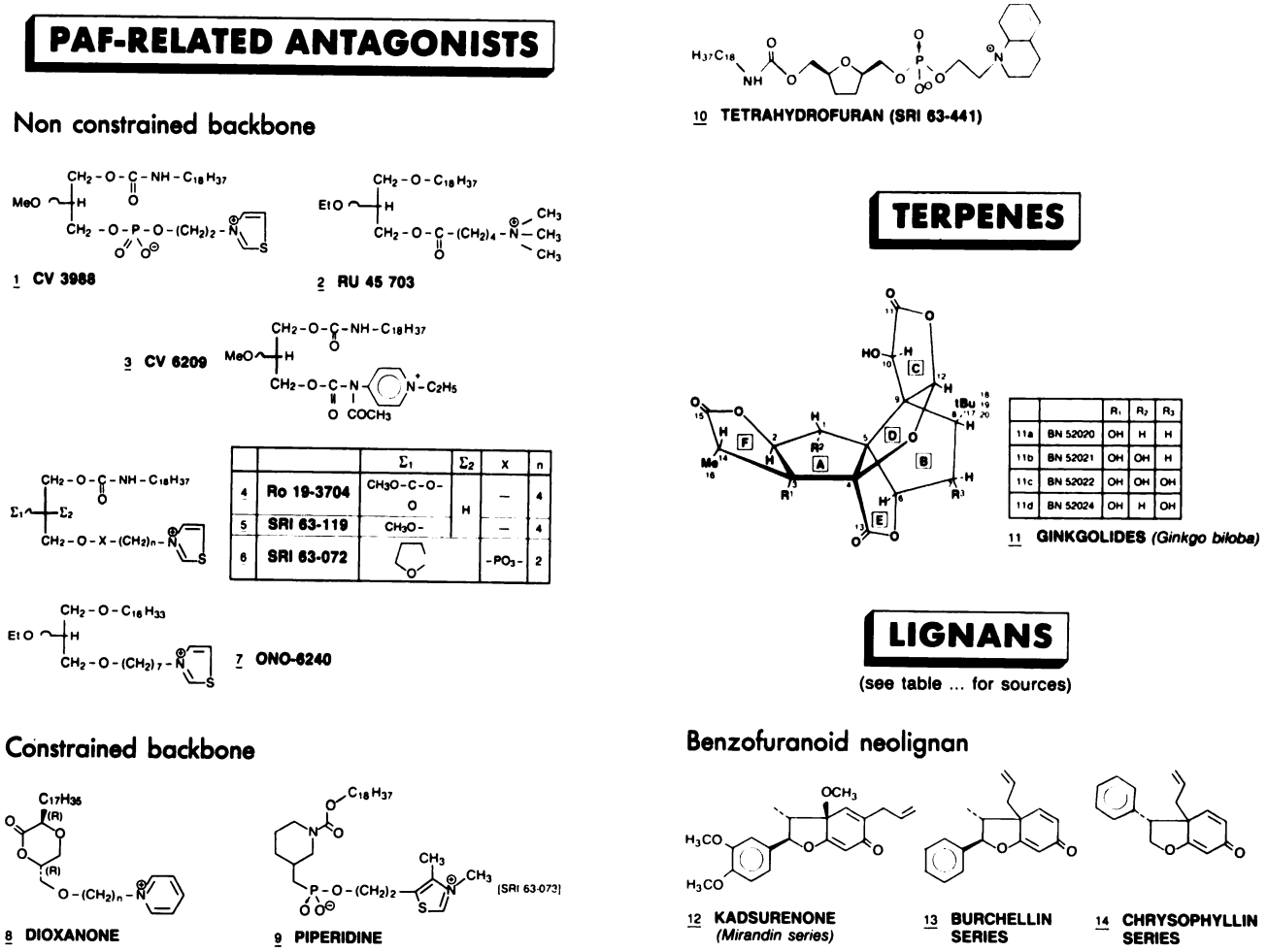
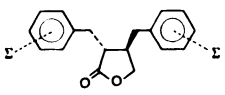


FIG. 13. Chemical structures of PAF antagonists (except calcium channel blockers and triazolobenzodiazepines).

FIG. 13—*Continued*

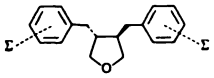
Dibenzylbutyrolactones (Butanolides)



15 **PRESTEGANE A, B**
MATAIRESINOL
EPIFs *(Endogenous PAF inhibiting factors)*

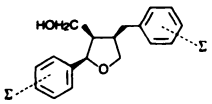
Substituted furans

• 3,4 - disubstituted furanoid lignans (type A)



16 **BURSERAN**

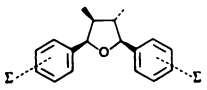
• 2,3,4 - trisubstituted furanoid lignans (type B)



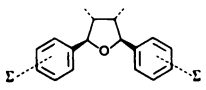
17 **LARICIRESINOL**

• 2,3,4,5 - tetrasubstituted furanoid lignans (type C) and related synthetic compound

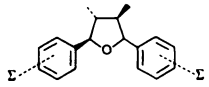
- natural



18 **(+) VERAGUENSIN SERIES**
2R-(2β, 3β, 4α, 5β)

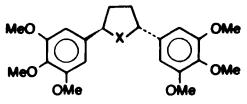


19 **GALGRAVIN (meso) SERIES**
2R -(2β, 3α,4α, 5β)



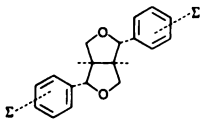
20 **(−) GALBEGIN SERIES**
2S-(2α, 3β, 4α, 5β)

- synthetic

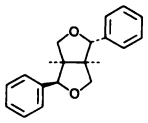


21 **L-652,731** (X = O)
22 **L-653,150** (X = S)
(X = O, S) *(dinor type C tetrahydrofuran lignan, 2α, 5β)*

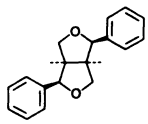
Substituted furofurans



23 **PINORESINOL SERIES**

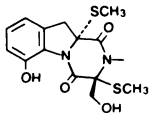


24 **EPIPINORESINOL SERIES**



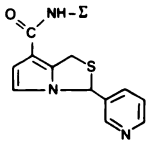
25 **EPIYANGAMBIN SERIES**

GLIOTOXIN



49 **FR-49175**
(Penicillium terlikowskii)

SYNTHETIC COMPOUNDS



27 **48740 RP** (Σ = H)
and related antagonists : **52629 RP** (Σ = )
52770 RP (Σ = )

tected rats from the development of i.v. PAF-induced extensive hemorrhagic lesions in the gastric mucosa. SRI 63-441 also attenuates endotoxin-induced lung injury in rats (198) and improves coronary flow during cardiac anaphylaxis (385b).

SRI 63-441 was also shown to act as a partial agonist in dogs allergic to *Ascaris suum* (434): the compound produced moderate bronchoconstriction and significant changes in the control of respiration by itself, and had no significant effect on the asthmatic reaction to allergen.

The two critical requirements to be achieved in this series are: (*a*) good oral absorption and (*b*) lack of partial agonism. Furthermore, the effects of these antagonists on enzymes of PAF metabolism (acetylhydrolase, acyltransferase) need clarification.

b. NATURAL PRODUCTS. The most promising chemical series of PAF inhibitors include natural compounds isolated from Chinese or Brazilian plants (terpenes and lignans) and from various bacterial strains (gliotoxines).

i. Terpenes. A family of potent PAF antagonists is formed by ginkgolides A, B, C, M, and J (respectively, BN 52020, BN 52021, BN 52022, BN 52023, and BN 52024; (fig. 13, 11) and terpenes isolated from the Chinese tree *Ginkgo biloba* L (62, 74) (reviewed in ref. 63). Ginkgolides are unique cage molecules which are C_{20} compounds, incorporating a tert-Bu group and six 5-membered rings including a spiro [4.4] nonane system, a tetrahydrofuran cycle, and three lactonic groups. Few studies had been devoted to these compounds since the pioneering structure elucidation of Nakanishi's group (298–300) and the discovery of the new ginkgolide J (504a). Recent studies using 500-MHz NMR and X-ray crystallography analysis (152) confirmed the structures assigned 15 yr ago by chemical methods.

BN 52021 and related antagonists inhibit PAF binding to rabbit (62, 74) and human (257, 261, 337) washed platelets and human leukocytes (62, 74). BN 52021 is the most efficient with an IC_{50} close to 10^{-7} M. Conversely, BN 52022 and BN 52024, which have an hydroxyl group in the vicinity of the *t*-Bu, are significantly less active, suggesting that the presence of a hydrophilic group close to the lipophilic moiety counteracts the antagonistic property. BN 52021 displaced [^{3}H]PAF from its receptor like nonlabeled PAF in both human (257, 337) and rabbit (74) platelets. The inhibitory activity is not removed by washing platelets (74, 337). BN 52021 is also an effective antiaggregating ginkgolide [IC_{50} (human, 7.5 nM PAF):

2.22×10^{-6} M; (rabbit, 2.5 nM): 1.88×10^{-7} M], and BN 52022 is the least efficient [IC_{50}: 1.83×10^{-6} M and 1.53×10^{-6} M, respectively] (BN 52020 $IC_{50} = 6.24 \times 10^{-6}$ M and 8.32×10^{-6} M, respectively) (74, 261, 337, 338, 405). This effect is specific to PAF, since no antagonism of other aggregating agents (ADP, collagen, AA, thrombin, A23187) was noted (74, 261, 337, 338, 405) and since BN 52021 does not inhibit AA metabolism nor the binding of a large variety of mediators [thromboxane A and its analog (U46619), leukotriene C_4, and others] (74).

Finally, a significant decrease in PAF-induced $[Ca^{2+}]_i$ measured by the fluorescent probe Quin-2 is recorded in rabbit platelets treated by BN 52021 (27, 424) which thus inhibits PAF binding to its receptor and the subsequent cellular response mediated by activation of the phosphatidyl inositol cycle and Ca^{2+} mobilization via a guanine nucleotide regulatory protein (17, 71a). In contrast, BN 52021 does not antagonize PMA-induced protein kinase C and phosphorylation (M. Castagna, personal communication). Interestingly, BN 52021 inhibits PAF-induced ion transport impairments in both macrophages (177) and guinea pig ventricle muscle fibers (139).

Platelet aggregation inhibition was also obtained ex vivo, since 6 h after p.o. administration of 5 mg/kg of BN 52021, the efficiency of PAF in aggregating platelets was significantly below that of the control group, demonstrating rapid p.o. absorption and a long-lasting effect.

BN 52021 inhibits PAF-induced aggregation and degranulation of human isolated neutrophils (74) and reduces chemotaxis induced by the autacoid (453, 502). It counteracts the PAF and opsonized zymosan-induced LTC_4 release by human eosinophils (77) and $O_2^{-\cdot}$ production by human (19, 222) and rabbit (71) PMN, guinea pig macrophages (142), and human eosinophils (77). In a similar way, ginkgolide B inhibits the thrombin-treated endothelial cell monolayers-enhanced $O_2^{-\cdot}$ generation by FMLP-stimulated polymorphonuclear leukocytes, suggesting a protective role in the oxidative stress of sepsis (510). This effect may result from the complete prevention by BN 52021 of the PAF-induced adhesion of labelled neutrophils to endothelial cells and the subsequent damages (492). Ginkgolide B also regulates beta-2 adrenergic receptor expression in the lung (5) and cerebellum (66). BN 52021 dose dependently inhibits in vitro PAF-induced adherence of human peripheral blood leukocytes from patients with ischemic heart diseases (167) and antagonizes the effects of PAF on human endothelial cells (81) and the related PGI_2 synthesis (145).

BN 52021 dose dependently inhibits the enhancement of cytotoxicity due to PAF on *Schistosoma mansoni* coated with both complement (C_{3b}) and specific antibody (IgG) (304). More interestingly, BN 52021 and the related ginkgolides decrease the IgE-mediated cytotoxicity of human eosinophils (M. Capron, personal communication). These data suggest that BN 52021 may interfere with the amplification of allergic reactions and inflammatory responses associated with helminthic infections. Its effects are consistently above those of BN 52020 and BN 52022 in all the models used, demonstrating that the effects are mediated by its action at the PAF receptor level.

In human lung tissue preparations, BN 52021 significantly counteracts the binding of [^{3}H]PAF to its receptor (233), with an $IC_{50} \simeq 10^{-9}$ M (5) in agreement with the inhibition of PAF-induced contractions of guinea pig lung parenchymal strips (142, 469, 470).

Finally, BN 52021 is also effective in complex models: ginkgolide B (*a*) inhibits PAF (23, 142, 400, 401)- or IgG (162, 400, 401)-induced hypotension, hemoconcentration, and extravasation in the rat, both preventively or curatively, and the related enzyme release (162); (*b*) antagonizes PAF-induced extravasation in the lung (26) and cerebral tissue (374) and the renal vascular tachyphylaxis to norepinephrine in the perfused rabbit kidney (174); (*c*) inhibits thromboformation induced by electric stimulation of the rat carotid artery (74) as well as the PAF-induced mesentery thrombosis in the guinea pig (59, 60); (*d*) normalizes some hemodynamic changes in experimental cirrhosis in the rat (495) and PAF-induced disturbances of renal tubular sodium transport (205, 372); (*e*) antagonizes tissue plasminogen activation induced by PAF (157); (*f*) counteracts preventively and curatively the hypotension induced by *Salmonella enteritidis* in the rat (158, 159) or by *S. typhimurium* in the guinea pig (3, 4)—mycotoxins (trichotecene) in rats (163)—as well as tissue plasminogen activation; a similar beneficial effect is observed in rats treated with trichothecen mycotoxin (163), this effect being correlated with a decrease in endotoxin-induced platelet accumulation (30) and hyperglycemia (A. Etienne and P. Braquet, in preparation); (*g*) antagonizes specifically PAF (253, 366, 436)- or antigen (42, 253, 366, 436, 522)-induced coronary vasoconstriction in the isolated guinea pig heart; (*h*) prolongs experimental cardiac allograft survival in the rat (170, 171, 249); (*i*) antagonizes PAF (i.v. or aerosol)-induced bronchoconstriction (62, 68, 142) and heterologous (62, 68, 470) or homologous (264, 494) passive bronchospasm (reviewed in ref. 71) in the guinea pig, antigen being administered i.v. or by aerosol (125). This protective effect is correlated with: a significant antagonism of PAF-induced airway vascular permeability (160); a concomitant dose-dependent decrease of the release of TxB_2, PGE_2, and LTC_4 in both PAF (142)- and ovalbumin (201)-challenged isolated lungs; and a significant inhibition of PAF-induced pulmonary hypertension and edema in isolated perfused lungs (235). Interestingly, Arnoux and Gillis (16) had shown that BN 52021, if given not more than 40 s after challenge, reverses the effects of PAF in isolated perfused rabbit lung. BN 52021 may also block the antigen-induced active shock in guinea pigs (41), but this has not been always confirmed. Indeed, the ginkgolide B may be less or not effective when low-sensitizing doses of antigen are used, a model in which anti-H1 is effective (C. Touvay and S. Des-

quand, unpublished; reviewed in ref. 71). The protection exerted by BN 52021 may be related to the significant decrease in eosinophil infiltration observed in treated animals after PAF or antigen challenge (C. Page, personal communication; A. Lellouch-Tubiana, personal communication). Interestingly, ginkgolide B is also effective against increased airway reactivity in both ragweed-challenged sensitized rabbits (133b) and in antigen (aerosol)-challenged actively sensitized guinea pigs (467) (fig. 11); (*j*) significantly antagonizes PAF-induced impairments of glomerules (138) and reduces in rats acute renal failure induced by immune challenge (153), glycerol (287a), and Adriamycin (anthracyclin-induced nephropathy) (153). In the latter model, ginkgolide B also abolishes lethality induced by Adriamycin; (*k*) protects the rat gastric (151a) and intestinal (C. Tagesson and D. Lewis, in preparation) mucosa against ischemic damages; (*l*) inhibits the UV-induced killing of *Candida albicans* by human keratinocytes in culture (A. Dobozy, personal communication), suggesting an antianaphylactoid effect in skin; (*m*) inhibits the deleterious effects of PAF on electroretinographic waves in rat isolated retina (151); (*n*) moderately antagonizes corneal edema and neovascularization of the immune keratitis and significantly accelerates corneal wound healing in rabbits (491a). Furthermore, BN 52021 accelerates shedding and phagocytosis of rod outer segments in frog eyecups (N. Bazan, personal communication) and inhibits the transient increase in intraocular pressure induced by laser iridal burns (491a); (*o*) antagonizes the gastric and intestinal ulcerations induced by PAF and endotoxin in the rat (67, 499) (fig. 12); (*p*) inhibits specifically rat paw edema induced by PAF (133). Interestingly, ginkgolide B [10 mg/kg, i.p. (but not 5 or 20), 30 min prior challenge] partially inhibited *Bothrops jararaca* venom-induced rat paw edema (470a); (*q*) antagonizes the PAF-induced impairments of atrial natriuretic factor effects in dogs (458, 459) and potentializes the hypotensive effect of captopril (P. Braquet and J. Baranes, unpublished); (*r*) antagonizes fibrin deposition and fibrinoid reaction after microporous urethane prosthesis implanted in pigs (296a); (*s*) significantly reduces the stroke index after unilateral ligation of the carotid artery in gerbils (B. Spinnewyn and P. Braquet, in preparation); and (*t*) significantly increases insulin release from isolated Langherans islets (G. Farkas, personal communication).

BN 52063 (a standardized mixture of ginkgolides A, B, and C) is also a potent inhibitor of PAF effects in humans: it dose dependently antagonizes both wheal and flare (124, 188a) and ex vivo platelet aggregation induced by the autacoid (124). No side effects were reported, even with high doses (124, 188a). Preliminary results obtained in randomized double-blind studies have shown that BN 52063 (120 mg p.o., 2 h before challenge) 'sly antagonizes immediate and delayed hypersensitivity in asthmatic patients challenged with specific antigen and acetylcholine (P. Guinot, personal communication).

ii. Lignans. Lignans and neolignans are an immense chemical family formed biogenetically by oxidative dimerization of hydroxyalkoxyphenylpropane (381). The term lignan is traditionally reserved for compounds in which the precursors are linked by β-carbons of each lateral chain, whereas the term neolignan is applied to products in which dimerization intervenes on carbons other than β.

Benzofuranoid neolignan. A neolignan isolated by the Merck group from *Piper futokadsurae* (haifenteng, piperaceae), a plant used in Southern China as antirheumatic and antiallergic, was the first natural product discovered as a potent inhibitor of the binding of [^{3}H]PAF to a rabbit platelet membrane preparation with an $IC_{50} = 10^{-7}$ M and $K_i = 5.8 \times 10^{-8}$ M (111a, 417). It was named kadsurenone (fig. 13, 12) and was shown to be a specific and potent inhibitor of PAF-induced platelet aggregation (e.g., $IC_{50} = 9.94 \times 10^{-7}$ M rabbit PRP). This result is correlated with the potent effect of kadsurenone in inhibiting the binding of [^{3}H]PAF on both its platelet and lung receptors (180, 184). The chemical specificity of kadsurenone was demonstrated by the weak activity of several related compounds isolated from the same plant (e.g., kadsurin A, kadsurin B, and piperenone) and several synthetic analogs with altered stereochemistry or ring substituents. Only the dihydro derivative, obtained by hydrogenation of the allyl side chain, retained full activity (230, 376). The inversion of the configuration at either the 2- or 3a-position results in a significant decrease of the receptor inhibitory activities. Furthermore, the 6-oxo group appears fundamental for activity, since the related methoxyimino or alcohol analogs are weak inhibitors. Interestingly, desallylkadsurenone is also weakly active, indicating the contribution of a lipophilic allyl or propyl side chain at the 5-position (69, 179, 376).

Kadsurenone inhibits PAF-induced aggregation and degranulation of human neutrophils (417). In a Langendorff preparation of isolated guinea pig heart perfused at constant pressure, kadsurenone (1 μM) effectively antagonized the PAF-induced decrease in contractility and coronary flow (282).

The neolignan is also active in blocking PAF-induced cutaneous permeability in the guinea pig (234). It inhibits PAF-induced increases of hematocrit and circulating N-acetylglucosaminidase in the rat at doses above 10 mg/kg i.p. and cardiovascular changes in the rabbit. Finally kadsurenone partially antagonizes endotoxic shock in rats (149).

However, kadsurenone has a relatively short duration of action, with $t_{1/2}$ from 30 min to 2 h in animals. In a metabolism study in rhesus monkeys, it was demonstrated that the alkyl side chain was oxidized and formed glucoronides (459a).

Gottlieb et al. (186a) recently extended the structure-activity relationships in the benzofuranoid series: generally the displacement of the allyl chain on position 8 of the benzofuranoid framework [burchellin (fig. 13, 13)

and chrysophyllin (fig. 13, 14) derivatives] decreases activity. A similar result is obtained by changing the position of the aryl group from C_2 [mirandin series (fig. 13, 12) and burchellin (fig. 13, 13) series] to C_3 [chrysophyllin series (fig. 13, 14)]. In agreement with these considerations, megaphone, a benzofuranoid lignan extracted from *Aniba megaphylla*, displays only a moderate activity (A. Esanu and P. Braquet, unpublished).

Substituted furanoid lignans. Several structures (45, 46) in lignan series are also potent PAF antagonists: this is especially the case with tetrahydrofuran-derived compounds, type C (2,3,4,5-tetrasubstituted) in Haworth's classification (table 5 for details): Veraguensin (46) (fig. 13, 18; $IC_{50} = 1.1 \times 10^{-6}$ M). Galbegin (69) and Galgravin (69) (fig. 13, 20, 19; $IC_{50} = 1.2 \times 10^{-6}$ M and 0.6×10^{-6} M, respectively) (69), respectively isolated from *Magnolia acuminata* (USA) and *Himantandra belgravena* (New Guinea), are relatively potent and specific inhibitors of PAF-induced rabbit platelet aggregation. Similar results were obtained with some guaiacin derivatives isolated from *Guaiacum officinale* L. or *Guaiacum sanctum* L. (Zygophyllaceae) or nectandrin A and nectandrin B isolated from the Brazilian *Nectandra rigida* (69).

Systematic synthetic study from the natural tetrahydrofuran framework led to the synthesis of a dinor type C tetrahydrofuran lignan, L-652,731 (fig. 13, 21), which is orally active and several times more potent than kadsurenone in vitro and in vivo (229). It inhibits [^{3}H]PAF binding with an $IC_{50} = 1.9 \times 10^{-8}$ M ($K = 9.8 \times 10^{-9}$ M) and is a very potent inhibitor of PAF-induced rabbit and human platelet aggregation. L-652,731 inhibits PAF (10^{-6} M)-induced human neutrophil degranulation ($IC_{50} = 3.6\ 10^{-7}$ M) (229). It reverses hypotension, extravasation, neutropenia, and release of lysosomal enzymes in rats by either i.v. or p.o. administration. It also counteracts similar changes induced by soluble immune complexes (148) and in endotoxemia (150) (5×10^{-8} M) as well as the edema in a reversed passive Arthus-type allergic inflammation in the rabbit (234).

In the type C tetrahydrofuran series, the *trans* isomers are generally more potent than the corresponding *cis* isomers (46). Aromatic ring substitutions are also relatively specific, the decrease in OMe substitution often leading to a decrease in activity (229). Nevertheless, the bis(3,4,5-trimethoxy phenyl) structure is activity enhancing but not essential.

Recently, the thioisostere of L-652,731 [L-653,150 (fig. 13, 22), *trans*-2,5-bis(3,4,5-trimethoxyphenyl)tetra-

TABLE 5

PAF-antagonist activity of various lignan series [P. Braquet, in preparation with the exception footnoted].

Lignan series	Compound	Source	IC_{50} (μM)*
Tetralines	Podophyllotoxin	Podophyllum sp.	>1000
	Peltatin	*Taiwana cryptomeriodides*	
	Taiwanin C		
Dibenzocyclooctadienes	Stegane	*Steganotaenia araliacea*	>1000
	Steganone		
Dibenzylbutyrolactones (butanolides)	Enterolactone	Mammalian urines	800
	OMe enterolactone	Mammalian urines	700
	Prestegane A	*Steganotaenia araliacea*	141
	Prestegane B	*Steganotaenia araliacea*	107
	Matairesinol	Abies sp.	243
	Thujaplicatin	*Thuja plicata*	427
Substituted furans			
3,4-disubstituted furanoids (type A)	Burseran	*Bursera microphylla*	98
2,3,4-trisubstituted furanoids (type B)	Lariciresinol	*Larix decidua*	320
2,3,4,5,-tetra substituted (type C)	Veraguensin	*Magnolia acuminata*	1.1†
		Ocotea veraguensis	
		Trimenia papuana	
	Calopiptin	*Pipto calix moorei*	1.0
		Magnolia acuminata	
	Galbegin	*Himantandra belgravena*	0.6
	Grandisin	*Litsea grandis*	0.9
	Galgravin	*Himantandra belgravena*	1.2
Substituted furofurans	Pinoresinol	Pinus, Abies sp.	197
	Sesamin	*Ginkgo biloba*	304
	Episesamin	*Zanthoxylum acanthopodium*	17
	Fargesin	*Magnolia fargesii*	1.2†
	Epiyangambin	*Viriola peruviana*	284

* Rabbit platelet, PAF (2.5 nM).

† Results from ref. 357.

hydrothiophene] was synthesized (45, 419). It inhibits binding of [^{3}H]PAF-acether to isolated rabbit platelet membranes with an IC_{50} of 1.9×10^{-8} M and 5-lipoxygenase-catalyzed transformation of [^{14}C]arachidonic acid to 5-HETE with an IC_{50} of 1.9×10^{-8} M (45, 419). The higher efficacy and longer duration of action of L-653,150, when compared to L-652,731, were demonstrated in a variety of models including PAF-induced human neutrophil degranulation and aggregation, rabbit platelet aggregation, guinea pig neutrophil aggregation in vitro and PAF-induced enzyme release, vascular permeability, and hypotension in vivo (45, 418).

Burseran (fig. 13, 16), a type A 3,4-disubstituted furanoid lignan isolated from *Bursera microphylla* (Burseraceae), is a moderate specific inhibitor of PAF-induced rabbit platelet aggregation (69). Lariciresinol (fig. 13, 17), a type B 2,3,4-trisubstituted furanoid lignan isolated from *Larix decidua*, is also a mild inhibitor of [^{3}H]PAF binding to its receptor (P. Braquet, unpublished).

Dibenzylbutyrolactone (butanolide)-derived lignans. Butanolide lignans (fig. 13, 15) are isosteres of type A furanoid lignans. In this series, presteganes A and B and various methoxylated matairesinols isolated from *Steganotaenia araliacea* are moderate antagonists (65).

Lignans of this series were recently discovered in animal urines (including human) (414, 440). They are characterized by the presence of one meta OH-group in each aryl ring, the main lignan identified in mammalian rings being enterolactone. Methyl derivatives were found only in limited quantities. These mammalian lignans are weak inhibitors of PAF-induced rabbit platelet aggregation ($IC_{50} = 10^{-6}$ to 10^{-4} M) (65). Interestingly, they are also inhibitors of the Na^+, K^+ pump in both human erythrocytes and human and guinea pig hearts (73). Since lignan concentrations in urine increase during early pregnancy, they might be chemically related to endogenous PAF inhibitors (and also to endogenous pump inhibitors), acting as a negative feedback against PAF and plasminogen activator which could be released in this state.

Substituted furo[3,4-*c*]furanoid lignans. In general, substituted furofuranoid lignans belonging to the pinoresinol series (fig. 13, 23), epipinoresinol series (fig. 13, 24), and epiyangambin series (fig. 13, 25) are moderate or weak inhibitors of PAF-induced rabbit platelet aggregation (P. Braquet, unpublished; table 5). Nevertheless, Pan et al. (357) found a relative potent inhibition with Fargesin ($IC_{50} = 1.2 \times 10^{-6}$ M), a lignan isolated from *Magnolia fargesii* with an epipinoresinol framework.

Other frameworks. Compounds from other lignan series {tetralines, dibenzo[4,5; 6,7]cyclooctadiene (steganederived compounds) and [3,4-*c*]furanoids} are inactive against PAF-induced rabbit platelet aggregation (P. Braquet, unpublished; table 5).

iii. Gliotoxin and related products. Very recently, PAF antagonists were found produced by the fermentation of different fungi and microorganisms (343–345). These products derive from bisdethiobis(methylthio) gliotoxin (fig. 11, 18) which was first isolated from the wood fungus *G. delquescens*.

Most of these products possess the dialkylthiopiperazinedione skeleton. The most potent antagonists are FR-900452 (*S. phacofaciens*) and FR-49175 (*P. tertikowskii*; fig. 13, 26): several analogs were also reported. These products inhibit PAF-induced rabbit platelet aggregation, but a slight inhibition of collagen-induced aggregation (but not of AA and ADP) was recorded.

Structure-activity relationships deduced from the *in vitro* platelet aggregation data (343) showed that the long-chain alkyl sulfide in the dialkylthiopiperazinedione system decreases the inhibitory activity.

The dihydrobenzene ring system does not seem to be essential for the activity, since the phenolic dihydro derivative still showed significant activity, and the anhydro compound showed higher inhibitory activity than the natural product. The hydroxymethyl group at position 3 appears to play an important role, since various dehydroxymethylated compounds were less active than the corresponding compounds, although some exceptions were found. As anticipated, the desulfurized compound lost the activity completely.

FR-49175 (0.1 mg/kg, i.v.) significantly inhibited PAF-induced bronchoconstriction in guinea pigs. However, it did not prevent PAF-induced hypotension in rats or vascular permeability increase in mice and failed to inhibit immune anaphylaxis in guinea pigs. These results suggest the possible existence of different types of specific receptor on platelets and the vascular wall (343–345). In contrast, FR-900452 is a potent inhibitor of endotoxin-induced thrombocytopenia (343a).

c. SYNTHETIC STRUCTURES. Very few products of synthetic origin have been described as having specific PAF-antagonistic properties. 48740 RP, a (3-pyridyl)-1*H*, 3*H*-pyrrolo[1,2-*c*]thiazole derivative (fig. 13, 27) synthesized for antiplatelet testing, was found to inhibit PAF-induced human and rabbit platelet aggregation ($IC_{50} = 6.9 \times 10^{-5}$ M and 3.3×10^{-6} M, respectively) (278, 411, 412). This inhibition is not selective, since 48740 RP interferes with platelet aggregation by AA, U 46619, collagen, and thrombin (0.05 units/ml) at high concentrations (1). 48740 RP is a competitive and full antagonist of PAF binding sites ($K_i = 2.3 \times 10^{-6}$ M) and inhibits PAF-induced hypotension, thrombocytopenia, hemoconcentration, and hyperfibrinolysis in the rabbit or in the rat at 10 mg/kg i.v. A protective effect against PAF (i.v.)-induced bronchospasm has also been observed in the guinea pig at 30 mg/kg p.o. 48740 RP protects against endotoxin-induced hemoconcentration and PAF-induced gastric ulcerations in the rat at 10 mg/kg p.o. (Floch et al., in preparation). Nevertheless, 48740 RP alone fails to inhibit passive homologous bronchospasm in guinea pigs (B. B. Vargaftig, unpublished) and allergic reaction in awake dogs (435). Two new PAF antagonists related

to the 48740 RP framework, 52629 RP and 52770 RP, have been recently described (110), their properties still being unknown.

In general, lipophilic antiinflammatory agents, such as indomethacin, sulindac, and ibuprofen, are not PAF antagonists (228). However, the modest activity of several indolyl and indenyl acetic acid derivatives led to the synthesis of a series of E-1-*p*-sulfamylbenzylidenyl indenes with an IC_{50} of 10^{-7} M in the receptor binding assay, the best compound in this group being E-1 [(4′-aminosulfonyl)phenyl] methylene-2-methyl-1*H*-indene-3-(2′-thiomethoxy)ethane. These compounds also inhibit PAF-induced cellular responses in platelets and human neutrophils and cutaneous inflammation in the rat and guinea pig (Yang et al., in preparation).

d. PHARMACOLOGICAL AGENTS. Products belonging to well-known pharmacological classes show selective antagonism against PAF.

i. Triazolobenzodiazepines. Triazolobenzodiazepines (TBDZ) are classical psychotropic agents. Kornecki et al. (255) have recently shown that alprazolam and triazolam potently inhibited PAF-induced human platelet activation. Other benzodiazepines, e.g., diazepam or chlordiazepoxide, are not active. These effects were specific to PAF, since the response of human platelets to ADP, thrombin, epinephrine, collagen, AA, and the calcium ionophore A23187 was not inhibited. Alprazolam and triazolam are not the most powerful compounds (IC_{50} = 2 to 12 × 10^{-6} M; 1 to 7 × 10^{-6}, respectively) in this series, since brotizolam, a thienotriazolobenzodiazepine (table 6), specifically inhibits PAF-induced human platelet aggregation with an IC_{50} of 5.4 × 10^{-7} M (101).

The structure-activity in this chemical series has been recently published by the Boerhinger research group (107–109, 503) (table 6 for details). The triazole ring is required for inhibition of PAF action; however, the presence of the triazole ring per se is not sufficient for PAF antagonism, since Kornecki et al. found that trazodone, a phenylpiperazine derivative of triazolopyridine, had an IC_{50} value of 10 μM and adinazolan, a triazolobenzodiazepine which contains a polar group (dimethylamino) at the C-1 position of the triazole ring, was ineffective as a PAF antagonist (255a).

This effect is also found in in vivo models using platelet aggregation continuously recorded by means of ^{111}In-labeled platelets in anaesthetized guinea pigs (101). Pretreatment (1 to 10 mg/kg p.o.) with brotizolam dose dependently inhibits the intrathoracic platelet accumulation of radioactivity induced by an i.v. infusion of PAF (30 mg/kg/min). This protective effect of brotizolam against PAF effects was confirmed in various models including lethality, bronchoconstriction, and hypotension (101), suggesting a possible relationship between the CNS effect and PAF antagonistic activity of TBDZ. This assumption was ruled out by the recent work of Casals Stenzel et al. (105, 106), who showed that the specific TBDZ antagonist, Ro 15-1788, does not interfere with the brotizolam-induced PAF inhibition, whereas the hypnogenic effect of TBDZ is totally suppressed by Ro 15-1788. The synthesis of a new TBDZ devoid of hypnogenic effect was recently accomplished by the introduction of a hydrophilic side chain to a thienotriazolodiazepine, WEB 2086 (103, 104, 503) (table 6), which inhibits PAF-induced human platelet and neutrophil aggregation in vitro with IC_{50} = 0.17 and 0.36 μM, respectively. Aerosols of WEB 2086 (0.25 to 0.5 mg/ml solution) also inhibit the bronchial and circulatory effects of i.v. PAF infusion in guinea pigs (104, 503). WEB 2086 is effective against several models of anaphylaxis and antagonizes endotoxin-induced shock and PAF-induced gastric ulcerations (102).

ii. Calcium channel blocking agents. Recent reports have shown that certain calcium channel blocking agents of class II, such as gallopamil (D 600), and class III (diltiazem) (472) are PAF inhibitors. Conversely, the products of class I (1,4-dihydropyridines) are only weak inhibitors (472). This inhibition appears to be stereospecific, since the (+)-*cis* isomer is 4.3-fold more potent than the (−)-*cis* isomer and is reversed by calcium (497a). A recent work by Valone (475) showed that inhibition of binding of [^{3}H]PAF by diltiazem and verapamil results from competitive and noncompetitive mechanisms. This effect is mediated by allosteric mechanisms similar to those described for calcium channel blockers and adrenergic receptors.

Recently, Hwang et al. (227) described a new dual antagonist 14-acetoxy-7β-(3′-ethylcrotonoyloxy)notonipetranone (L-652,469) isolated from the methylene chloride extracts of the buds of *Tussilago farfara* L. This compound inhibits the [^{3}H]PAF-specific binding to rabbit platelet membranes with equilibrium inhibition constants K_i of 0.56 and 6.0 μM in the presence or in the absence of 150 mM NaCl, respectively. It also inhibits the specific binding of Ca^{2+} channel blockers (e.g., [^{3}H] nitrendipine; K_i = 1.2 μM) in cardiac sarcolemmal vesicles.

VIII. PAF Receptors

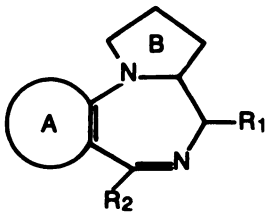
A. Presence and Characteristics of PAF Receptors

The involvement of specific receptor(s) was first suggested by the demonstration that only the naturally occurring stereoisomer (*R*) stimulated various PAF responses (214, 215, 481). Additional data corroborated these findings: very low concentrations (usually lower than 0.1 nM) are necessary to trigger biological effects; specific desensitization takes place after tissue exposure to PAF; and there is specific inhibition by PAF antagonists.

The existence PAF receptors has recently been confirmed by binding experiments using [^{3}H]PAF. High affinity receptors were found in human (230, 236, 251,

TABLE 6

PAF antagonistic property in triazolobenzodiazepine series: structure-activity relationships.



A	B	R_1	R_2	1*
		H		16
		H	Cl	8.6
Cl (Alprazolam)		H		14
Cl (Triazolam)		H	Cl	9
Cl		H	Cl	300
CH_3		H	Cl	9.1
S		H	Cl	22
O_2N S		H	Cl	2.6
O N–C–$(CH_2)_2$ S (WEB 2086)		H	Cl	1.3
Br S (Brotizolam)		H	Cl	4.3
Br S		H	Cl	5.4
S		H		19.8
S		H	Cl	7.6
S S		H	Cl	5.2
Br S	H	H	Cl	56
N S CH_3	H	H		700
C_2H_5 N N CH_3		H	Cl	270
CH_3–N N		H	Cl	ND
CH_3 N N N		H	Cl	950

1* = IC_{50} (μM)

252, 476) and rabbit (62, 74, 228–230, 232) platelets, human neutrophils (477), and human lung membrane (5, 233) (table 7). The affinity and number of these receptors are interrelated with the tissue and species specificity.

Rat platelets are not aggregated by PAF in vitro. This was explained by the fact that they do not have high affinity receptors (236). The K_d values for platelets are approximately 10 to 100 times greater than the concentrations of PAF giving half-maximal aggregation. Full occupancy of the receptors may thus not be necessary for a maximal aggregation response. Furthermore, the affinity of PAF binding is 10^5 times higher than for ADP (270) and 4 to 20 times higher than for thrombin (422), if the number of human platelet receptors is about one order of magnitude smaller than those of several hormones. Conversely, human PMNLs present a relatively high number of sites explaining the effect of PAF on these cells.

TABLE 7
Occurrence and characteristics of PAF receptors.

Material	K_d ($\times 10^{-9}$ M)	No. of sites/cell	Ref.
Human PMN	0.11 ± 0.02*	$5.2 \pm 2.1 \times 10^6$	477
Human lung	0.49 ± 0.17	140 ± 7 fmol/mg protein	233a
Human platelet	37 ± 13	1,399 ± 498	476
Human platelet	1.58 ± 0.36	1,983 ± 391	236
Human platelet	0.053 ± 0.014	242 ± 64	252
Human platelet	0.78	150–300 ($1.61 \pm 0.34 \times 10^{12}$ mg of membrane)	233
Rabbit platelet	0.9 ± 0.5	19,386 ± 6,588	236
Rat platelet		Not found	236
Rat retina	0.77 ± 0.21		M. Doly & P. Braquet (in preparation)
Guinea pig endothelium	12 ± 7		P. Braquet (unpublished)
Guinea pig lung	0.27 ± 0.12		P. Braquet (unpublished)

* Mean ± SE.

The PAF receptor in platelet plasma membrane is heat labile and protease sensitive (233a, 474). In addition, when platelets are exposed to PAF at 37°C for 5 min, this leads to desensitization and a decrease in specific binding and aggregation (265). Platelets desensitized to PAF may have a normal response to other agonists such as ADP, collagen, thrombin, A23187, and arachidonic acid, which suggests that a specific receptor has been desensitized (113). Since there is no correlation between the effects of PAF on platelet aggregation and various physical properties of pure dipalmitoylphosphatidylcholine bilayer as detected by differential scanning calorimetry (233a), this suggests that PAF receptor sites may not be a phospholipid. Using chromatography of membrane preparation on a Sepharose column loaded with PAF/human serum albumin, Valone (474) attempted to characterize the high affinity PAF binding site present in human platelets. In an unconfirmed experiment, SED polyacrylamide gel electrophoresis of the eluted material revealed a single protein with an apparent molecular weight of 180,000. More recently, Nishihira et al. (335a) isolated PAF receptor from human platelets and identified it as a protein with a molecular weight of 160,000.

Unfortunately, more detailed biochemical characterization of the PAF receptor is hampered by the lack of a consistent procedure to solubilize the membrane binding protein and to prepare high titer specific antibodies to PAF (334).

Monovalent and divalent cations and GTP regulate the specific binding of PAF to platelet plasma membranes. Hwang et al. (230, 232) stated that inhibition of [^{3}H]PAF binding is sodium specific, with a 50% effective dose (ED_{50}) = 150 mM. In contrast, K^+, Cs^+, and Rb^+ and the divalent cations Mg^{2+}, Ca^{2+}, and Mn^{2+} enhance the binding (232). It is interesting to note that binding of catecholamines to α-adrenergic receptors is enhanced by GTP and Na^+ but not by K^+ (473).

A total inhibition of PAF-induced aggregation is observed when H_2O is replaced by D_2O in the incubation medium, although binding was not affected (233a). (*R*)-PAF stimulates hydrolysis of GTP (ED_{50} 10^{-9} M), whereas the nonnatural (*S*)-enantiomer is inactive up to 10 μM (17, 71a, 232). This suggests that the receptor may be linked to the adenylate cyclase system via an inhibitory guanine nucleotide regulatory protein (71a, 232).

As for the interaction between platelet and coagulation proteins, it is of interest to observe that binding of PAF to its receptor unmasks the glycoprotein IIb-IIIa complex, binding sites for fibrinogen, responsible for the platelet-to-platelet interaction (252). The PAF-induced fibrinogen binding is specific, saturable, and dependent on the cyclooxygenase pathway (PGs-endoperoxides-TxA_2) as well as ADP pathway (252). Finally, in experiments with a smooth muscle cell line, it was observed that PAF and related analogs impair the binding of [^{3}H] dihydroergocryptine to α-adrenergic receptors (428).

B. Putative Conformation of PAF Membrane Binding Sites

A putative conformation of PAF platelet membrane binding sites can be deduced on the basis of the data obtained with agonists and antagonists (70, 179). On the one hand, agonistic activity decreases when the fatty chain is shortened, whereas the introduction of a polar group close to the allyl moiety in kadsurenone and *t*-butyl in BN 52021 (e.g., BN 52022) greatly reduces antagonism. A lipophilic moiety thus seems essential for both agonistic and antagonistic activities, meaning that the long fatty chain of PAF deeply enters the membrane in a hydrophobic area (e.g., hydrophobic lipid-lipid or lipid-protein interactions; see fig. 4 for details). The anchorage of the chain in the membrane and the relative position of the ether function as compared with its environment certainly change membrane fluidity and metabolism. Membrane activation may possibly derive from

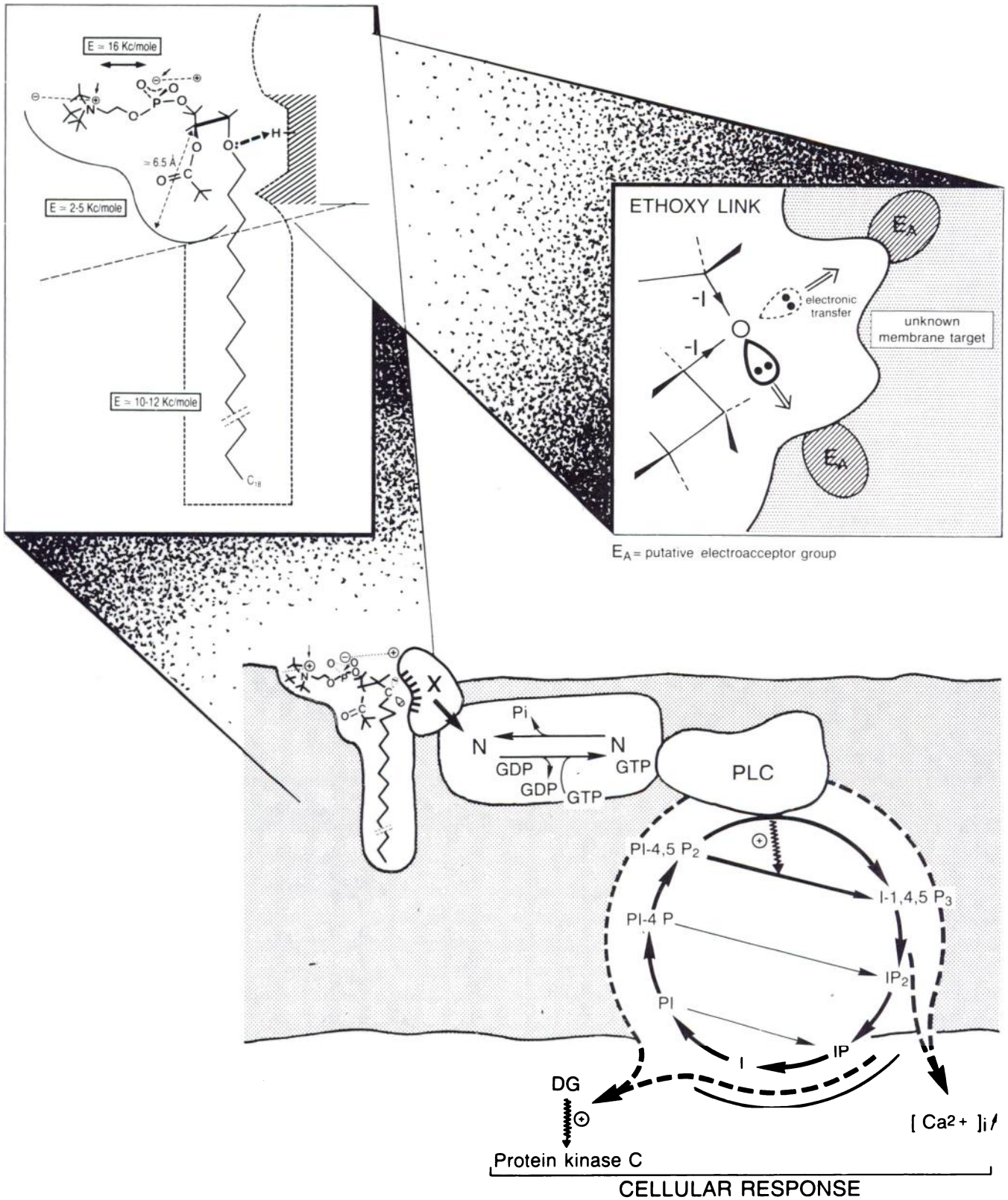


FIG. 14. Putative conformation of PAF-receptor and PAF-induced membrane signal transduction.

an electronic transfer from oxygen doublets of the ether function to an unknown membrane target. Thus, the low activity of the thioether derivative of PAF could be explained by the lower electronegativity of sulfur (2.5 versus 3.5 for oxygen) leading to a lesser availability of the doublets borne by the heteroatom. Analogs bearing an isosteric group like CH_2 which do not comprise doublets are inactive.

TABLE 8
Glossary of generic and chemical names

AA	Arachidonic acid
ADP	Adenosyl diphosphate
ALP:	Alkyllysophospholipids
ANF	Atrial natriuretic factor
Az	Azathioprine
A23187	Calcium ionophore A23187
BpB	Bromophenacyl bromide
BM-14-440	7-Ethoxy-4-hydroxy-N,N,N-trimethyl-3,5-dioxa-9-thia-4-phosphaheptacosan-1-ammonium hydroxide, inner salt, 4-oxide
BN 52020	3-*t*-Butyl-hexahydro-4,7*b*-dihydroxy-8-methyl-9*H*-1,7*a*-epoxymethano-1*H*,6*aH*-cyclopenta[*c*]furo[2,3-*b*]furo[3′,2′:3,4] cyclopenta[1,2-*d*]furan-5,9,12(4*H*)trione
BN 52021	3-*t*-Butyl-hexahydro-4,7*b*,11-trihydroxy-8-methyl-9*H*-1,7*a*-epoxymethano-1*H*,6*aH*-cyclopenta[*c*]furo[2,3-*b*] furo[3′,2′:3,4]cyclopenta[1,2-*d*]furan-5,9,12(4*H*)trione
BN 52022	3-*t*-Butyl-hexahydro-2,4,7*b*,11-tetrahydroxy-8-methyl-9*H*-1,7*a*-epoxymethano-1*H*,6*aH*-cyclopenta[*c*]furo[2,3-*b*] furo[3′,2′:3,4]cyclopenta[1,2-*d*]furan-5,9,12(4*H*)trione
BN 52023	3-*t*-Butyl-hexahydro-2,4,11-trihydroxy-8-methyl-9*H*-1,7*a*-epoxymethano-1*H*,6*aH*-cyclopenta[*c*]furo[2,3-*b*] furo[3′,2′:3,4]cyclopenta[1,2-*d*]furan-5,9,12(4*H*)trione
BN 52024	3-*t*-Butyl-hexahydro-2,4,7*b*-trihydroxy-8-methyl-9*H*-1,7*a*-epoxymethano-1*H*,6*aH*-cyclopenta[*c*]furo[2,3-*b*] furo[3′,2′:3,4]cyclopenta[1,2-*d*]furan-5,9,12(4*H*)trione
BN 52063	A mixture of BN 52020, BN 52021, and BN 52022 (40:40:20)
cAMP	Cyclic adenosine monophosphate
CDP	Cytidine diphosphate
cGMP	Cyclic guanosine monophosphate
CPG	Choline phosphorylglyceride
C3a	Activated complement factor 3
C5a	Activated complement factor 5
874 CB	α,β-Dibromo-3-chloro-4-cyclohexyl-γ-oxo-benzenebutanoic acid
CsA	Cyclosporine
CV 3988	3-(N-*n*-Octadecylcarbamoyloxy)-2-methoxy) propyl-2-thiazolioethyl phosphate
CV 6209	2-[N-Acetyl-N-(2-methoxy-3-octadecyclarbamoyloxypropoxycarbonyl)aminomethyl]-1-ethylpyridinium chloride
DAG	Diaclyglycerol
DFP	Diisopropyl fluorophosphate
EPF	Early pregnancy factor
EPIF	Endogenous PAF-inhibiting factor
ET-18-OCH_3	(±)-1-Octadecyl-2-methyl-glycero-3-phosphocholine
FMLP	N-Formylmethionylleucyl phenylalanine
FPL-55712	7-[3-4-Acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylic acid monosodium salt
FR-49175	[3*R*-(3α,5aβ,6β,10aα)]-2,3,5*a*,6,10,10a-hexahydro-6-hydroxy-3-hydroxymethyl-2-methyl-3-10*a*-bis(methylthio)pyrazino[1,2-*a*]indole-1.6-dione
FR-900452	FR-900452 has been isolated from *Streptomyces phaeofaciens* matsuenensis no. 7739 by Fujisawa. [CAS: 101706-33-6]
GPC	Glycerophosphocholine
GPE	Glycerophosphoethanolamine
HETE	Monohydroxyeicosatetraenoic acid
IL-1	Interleukin-1
IL-2	Interleukin-2
IP_3	Inositol 1,4,5-triphosphate
LTB_4	Leukotriene B_4
LTC_4	Leukotriene C_4
LTD_4	Leukotriene D_4
L-648,611	2-[N-Palmitoylamino]propylphosphocholine
L-652,469	14-Acetoxy-7β-(3′-ethylcrotonoyloxy)notonipetranone
L-652,731	*trans*-2,5-Bis(3,4,5-trimethoxyphenyl)tetrahydrofuran
L-653,150	*trans*-2,5-Bis(3,4,5-trimethoxyphenyl)tetrahydrothiophene
Ono 6240	1-O-Hexadecyl-(2*R*,*S*)-O-ethyl-3-O-(7-thiazolioheptyl)glycerol chloride
PAF	Platelet-activating factor
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PGE	Prostaglandin E
PGI	Prostaglandin I
PHA	Phytohemagglutinin
PI	Phosphatidylinositol
PKC	Protein kinase C
PLA_2	Phospholipase A_2
PMA	Phorbol myristate acetate

TABLE 8—*Continued*

PMN	Polymorphonuclear leukocytes
PMSF	Phenylmethylsulfonyl fluoride
PR 1501	*rac*-1-O-Octadecyl-2-acetamido-2-deoxylglycerol-3-phosphocholine
PR 1502	*rac*-1-O-Octadecyl-2-acetamido-2-deoxyerythrol phosphocholine
Ro 15-1788	Ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate
Ro 18-7953	3-{4-[2-Methoxy-3-(octadecylcarbamoyloxy)propoxy]-4-oxobutyl}thiazolium
Ro 18-8736	3-{4-[2-(Methoxycarbonylamino)-3-(octadecylcarbamoyloxy)propoxy]-4-oxobutyl}thiazolium
Ro 19-1400	3-{4-[2-(Methoxycarbonyloxy)-3-(octadecylcarbamoyloxy)propoxy]-4-oxobutyl}thiazolium
Ro 19-3704	3-{4-[(*R*)-2-(Methoxycarbonyloxy)-3-(octadecylcarbamoyloxy)propoxy]butyl}thiazolium iodide
48740 RP	3-(3-Pyridyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-7-carboxamide
52629 RP	N-(3-Methoxyphenyl)-3-(3-pyridinyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-7-carboxamide
52770 RP	N-(3-Chlorophenyl)-3-(3-pyridinyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-7-carboxamide
RU 45703	N,N,N-Trimethyl-N-(4-carboxybutyl)ammonium iodide, (2-ethoxy-3-octadecycloxy)propyl ester
SaRI-62436	(Not disclosed)
SaRI-62586	(Not disclosed)
Sin-1	3-(4-Morpholinyl)sydnone imine
SRI 62-834	(Not disclosed)
SRI 63-072	(*R,S*)-3-{2-[(2-Octadecylaminocarbonyloxymethyltetrahydro-2-furanylmethoxy)hydroxyphosphinyloxyethyl]thiazolium hydroxide, inner salt, 4-oxide}
SRI 63-073	3,4-Dimethyl 5-[2((1-octadecyloxycarbonyl-3-piperidinyl)methyl-hydroxy phosphinyloxy)ethyl]thiazolium, inner salt
SRI 63-119	(*R,S*)-3-{4-[(3-Octadecylaminocarbonyloxy-2-methoxy)propoxy]butyl}thiazolium bromide
SRI 63-441	*cis*(±)-1-[2-[Hydroxy[tetrahydro-5-[(octadecylaminocarbonyl)oxy]methyl]furan-2-yl]methoxyphsophinyloxy ethyl]quinolinium hydroxide, inner salt
TBDZ	Triazolobenzodiazepine
TxA_4	Thromboxane A_2
TxB_2	Thromboxane B_2
U 46619	(15*S*)-Hydroxy-11α,9α-(epoxymethano)prosta-(5*Z*,13*E*)-dienoic acid
U 66982	1-O-Octadecyl-2-acetyl-*sn*-glycero-3-phosphoric acid 10′-trimethylammonium decyl ester
U 66985	1-O-Octadecyl-2-acetyl-*sn*-glycero-3-phosphoric acid 6′-trimethylammonium hexyl ester
WEB 2086	(3-[4-(2-chlorophenyl)-9-methyl-6*H*-thieno(3,2-*f*) (1,2,4)triazolo(4,3-*a*) (1,4)-thienodiazepine-2-yl]-1-(4-morpholinyl)-1-propanone)

Isosteres which possess doublets involved in a mesomerism and which are therefore not available present a similar result. The presence of doublets could be made necessary by a possible protonation from the active site. Inhibition of aggregation induced by PAF in D_2O corroborates this hypothesis.

As presented in fig. 6, agonistic activity can be produced with a wide variety of substituents on the carbon 2 of the glyceryl backbone. The length and bulk of the moiety are the main factors to be noted. Agonistic efficiency is considerably lesser with substituents with large steric hindrance. The short chain may thus take part in the anchorage of PAF on its receptor, resulting in better alignment of the polar head of the mediator with that of membrane phospholipids. This assumption is reinforced by the necessary (*R*)-configuration generally required for activity.

A putative conformation of the PAF binding site is proposed in fig. 14 on the basis of the above considerations (70, 179). After binding to its receptor, PAF might indirectly influence the conformation of the unknown target sited within the membrane by an electronic charge transfer from the ether function (see above), by modification of the fluidity around the part of the targets included in the bilayer, and/or by deranging the external protein-phospholipid polar head interactions. The unknown receptorial protein may, in turn, activate the guanyl nucleotide regulatory protein with GTP hydrolysis. Phospholipase C is then stimulated with phosphodiesterase cleavage of inositol phospholipids, especially phosphatidyl inositol-4,5-bisphosphate (PI-4,5-P_2) into inositol-1,4,5-triphosphate (I-1,4,5-P_3) which induces Ca^{2+} mobilization from is internal pools. Diacyl glycerol is also produced which activates protein kinase C. Both increased $[Ca^{2+}]_i$ and protein kinase C activation mediate cellular response. PAF antagonists, which inhibit PAF binding to its receptor, antagonize all the events of the signalling process.

Such a receptor model can accommodate several potent inhibitors if it is considered that: (*a*) L-652,731, BN 52021, and kadsurenone all incorporate a tetrahydrofuran ring. Tetrahydrofuran oxygen is more basic than the ether oxygen in PAF and therefore more likely to undergo protonation; (*b*) competition between the tetrahydrofuran ring of inhibitors and the ether function in PAF is sterically possible from studies performed by molecular modelling (70, 179). It may be surmised that once the inhibitor has become well positioned in the receptor site, the two doublets of the tetrahydrofuran ring may then interact with the unknown target, and the rigidity of these cyclic structures may prevent the activation of transmembrane events (70, 179). (*c*) The introduction of a polar group close to lipophilic moiety lessens the antagonistic activity as seen in the ginkgolide series (BN 52022 and BN 52024) and some derivatives of kadsurenone.

IX. Prospects

The involvement of PAF and similar phospholipids in different pathologies is a real possibility, but major uncertainties remain to be clarified as to whether its role is an accessory or a main one. It is also difficult to accept that formation of PAF alone can explain events as complicated as acute endotoxic shock or immediate hypersensitivity. Researchers in the field should consider the pitfalls of overestimating their own hopes, as was the case with previous concepts such as TxA_2 being the mediator of platelet aggregation or of allergic bronchoconstriction. In the present situation, essential information will come from the characterization of different PAF receptors, possibly in leukocytes, and from the clinical trials with available antagonists, on systems such as the local inflammation induced by PAF or by allergens, or on asthmatic patients refractory to conventional therapy including corticosteroids. It is clear that further expansion in research concerning pharmacologically active lipids such as PAF will extend to fields which were not thoroughly reviewed by us here, such as tissue invasion, including embryo implantation, CNS, cancer, or myocardial infarction. Even though PAF may not be THE mediator of the third pathway of platelet aggregation, its synergy with platelet activation by epinephrine may have important consequences for the understanding (and the therapy) of different forms of shock, including anaphylaxis during myocardial infarction. Its potential role in extrapulmonary allergic or immunological diseases is also an open field. In fact, PAF poses a very special challenge to biomedical researchers, in that it is a mediator in search of a role.

An intriguing and potentially very exciting aspect is that PAF, as defined in this review, may represent the forerunner of a complex family of structurally related phospholipid mediators. Recent studies by Pinckard et al. (504b) and others have demonstrated an extensive molecular heterogeneity of PAF-like substances; the biological properties of these new lipid metabolites are still being unraveled.

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