Biochemistry of Platelet-Activating Factor: A Unique Class of Biologically Active Phospholipids[1] (42839)

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I. Introduction

In the autumn of 1979, three independent laboratories (1–3) described the chemical structure of a new subclass of phospholipids that possessed biologic properties identical to platelet-activating factor (PAF) and an antihypertensive polar renal lipid (APRL). The chemical features of this unique bioactive phospholipid are an *O*-alkyl ether group at the *sn*-1 position, an acetate at the *sn*-2 position, and a phosphocholine moiety at the *sn*-3 position of the *sn*-glycerol moiety (Fig. 1). A number of reviews have provided a detailed account of earlier developments in the PAF (4–11) and APRL (10, 12) fields and, therefore, only significant historical events are highlighted in this section.

The antihypertensive activity of an extract from the renal medulla was originally observed in dogs with renoprival hypertension (13). This antihypertensive principle could also be extracted from renomedullary interstitial cells of rats and was initially referred to as ARH, an antihypertensive renomedullary hormone (14). However, further characterization of the polar nature of this lipid led to changing the name of ARH to APRL, the abbreviation for antihypertensive polar renomedullary lipid (15).

Within the same time frame of the APRL investigations, several articles (16–18) appeared which described the release of vasoactive amines from rabbit platelets in a reaction that required leukocytes from an immunized rabbit, an antigen, and platelets; the reaction, referred to as leukocyte-dependent release of histamine (LDHR), was considered as an immediate hypersensitivity-type reaction linked to allergic responses. Benveniste *et al.* (19) further characterized this soluble factor and identified IgE-stimulated basophils as the source of the substance responsible for LDHR and the aggregation of platelets; these workers called the bioactive substance PAF. The lipid nature and polar properties of PAF finally became apparent from chemical studies of its solubility characteristics, chromatographic behavior on silicic acid, and reactivity with lipases (20, 21).

The APRL and PAF fields ultimately merged when it was realized that the hypotensive (2) and platelet-activating (1, 3) properties of a specific phospholipid molecule shared an identical chemical structure. After the chemical structure of PAF was known, AGEPC (acetylglyceryl ether phosphocholine), PAF-acether (a contraction of acetate and ether), and alkylacetyl-GPC (alkylacetylglycerophosphocholine) were used as alternate terms for PAF; however, no standardized abbreviation has yet been established for PAF by the IUPAC-IUB Commission on Biochemical Nomenclature. Obviously, PAF is a misnomer since the activities of this biologically active phospholipid are so diverse. It should be noted that a neutral lipid, alkylacetylglycerols (Fig. 1), also is biologically active, albeit, at considerably higher levels (22, 23).

In 1980 enzymes were described for the biosynthesis and inactivation of PAF in a variety of tissues (24); detailed accounts of their properties (25–27) were published soon thereafter. During the same year firm proof for the chemical structure of a native PAF was provided when sufficient amounts of PAF were isolated from IgE-stimulated rabbit basophils to carry out well-documented chromatographic, chemical, and mass spectrophotometric analyses (28).

My brief overview of the PAF field emphasizes the current understanding of PAF metabolism and its regulation, particularly in the context of explaining the enzymatic sources of PAF in physiologic vs pharmacologic processes. However, in order to appreciate more fully PAF as a unique and diverse type of phospholipid mediator, I have also included brief accounts of the biologic properties, structural-functional relationships, antagonists, receptors, and mode of action of PAF as

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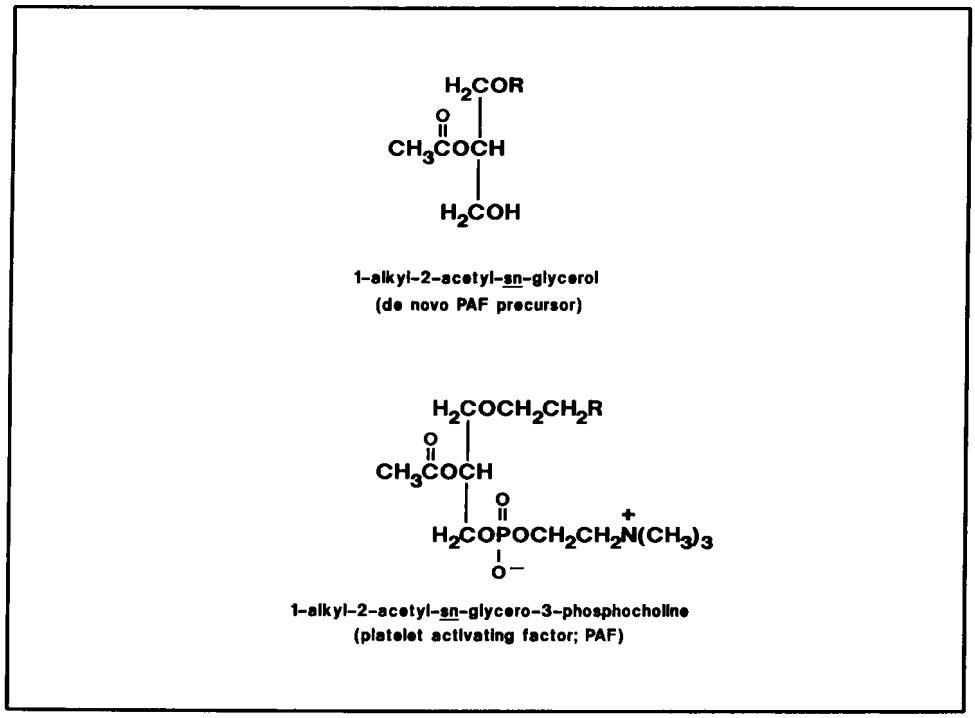

Figure 1. Chemical structure of platelet-activating factor and its biologically active precursor (1-alkyl-2-acetyl-*sn*-glycerol) in the *de novo* pathway of PAF biosynthesis.

well as implications of the involvement of PAF in specific diseases. The reader should consult the extensive reviews (4–11) and two recent books (29, 30) on PAF for broader coverage of this field.

II. PAF Production and Biologic Responses

It is beyond the scope of this minireview to discuss the extensive literature covering PAF biology. Furthermore, since this subject has been reviewed in-depth (4–11), only a few of the major bioactions of PAF described in studies of cellular, tissue, and *in vivo* systems are summarized in this section.

Many of the biologic effects of PAF or APRL were reported long before the discovery of the molecular structure of the phospholipid responsible. Anaphylaxis is the end result of the systemic events involving the complex interactions of IgE, basophils, PAF, and platelets. Under these circumstances platelets and neutrophils aggregate and undergo the accompanying release reactions that lead to bradycardia, cardiovascular, and respiratory collapse (4–11).

PAF can be produced in significant quantities by certain cells after exposure to appropriate stimuli. However, many cells have the capability of producing PAF without stimulation by *de novo* synthesis (see Part VA). Platelets and neutrophils not only are capable of producing PAF, but are also targets for PAF activation. In fact PAF itself can perpetuate its own synthesis in neutrophils (31). However, once cells are exposed to PAF, they become desensitized to PAF (32) (also see Part IV). In contrast, macrophages, endothelial cells, and HL-60 cells are sources of PAF but they do not appear to exhibit cellular responses similar to platelets or neutrophils when exposed to PAF (4–11, 29, 30).

Only sparse information is available about the nature of the molecular species of PAF produced under stimulated vs unstimulated conditions in different cell types. Hanahan *et al.* (28) showed that rabbit leukocytes containing IgE-sensitized basophils produced PAF consisting only of octadecyl (≃90%) and hexadecyl (≃10%) chains at the *sn*-1 position. Saturated C_{16} and C_{18} chains were also the principle homologs of lyso-PAF found in hog leukocytes (33). Analysis of stimulated human neutrophils by several groups led to conflicting conclusions about the nature of the molecular species of PAF produced. Mass spectrometric analysis by Clay *et al.* (34) indicated only a single species (16:0) of PAF was formed, whereas Oda *et al.* (35) detected both 16:0 and 18:0 species of PAF (in a ratio of 4:1). The mass spectra data of Pinckard and colleagues (36, 37) revealed at least four species of PAF in addition to the predominate hexadecyl type. Using high-performance liquid chromatography to determine the molecular species of labeled PAF formed from [^{3}H]acetate, Mueller *et al.* (38) found at least three PAF species were produced in addition to the major hexadecyl component in stimulated rabbit and human neutrophils. The *O*-alkyl chain in PAF has been reported to differ in stimulated vs resting human neutrophils (39); 16:0 predominated in A23187-stimulated cells, whereas significant amounts of 17:0, 18:0, and 18:1 alkyl moieties in addition to the 16:0 chain species were found in resting cells. Molecular

species of PAF also vary accordingly in the same cell types among different animal species (40). Molecular heterogeneity has also been observed in PAF species found in bovine brain (41) and in scaly lesions from patients with psoriasis (42). Differences in the molecular species composition of PAF may be explained on the basis of variations in the degree of cell activation, methodology used, and biologic factors (e.g., diet, drugs, etc.).

In studies of molecular species of PAF, it is important to keep in mind that the acyl analog of PAF can also be formed in appreciable quantities (41, 43, 44). The acyl analog of PAF possesses only slight, if any, PAF-like activity (see Part III), but the close structural similarity of this analog to PAF species can complicate the interpretation of molecular species data. An enzymatic basis for the formation of the acyl analog of PAF will be discussed in Part V of this review.

The physiologic significance of PAF species possessing different types of alkyl chains is unknown at the present time. Variations in the magnitude of the biologic potencies of different PAF species have been reported (see Part III), but differences in qualitative responses among various molecular species of PAF have not been documented.

III. Structural-Functional Relationships of PAF

Early investigations revealed that the chemical structure of PAF could not be modified to any great extent without losing its biologic activity (Table I). The original reports on the discovery of the PAF structure clearly demonstrated that the *sn*-2 propionyl analog was essentially equal to PAF with regard to both platelet (1) and blood pressure (2) responses, whereas the *sn*-1 acyl analog lacked both biologic activities. Importance of the three *N*-methyl groups at the *sn*-3 position of PAF was emphasized in experiments that showed the stepwise removal of the methyl groups diminished the biologic activity accordingly until all activity was abolished when the *sn*-3 phosphoethanolamine analog was tested for serotonin release from rabbit platelets (45). A number of publications have described the structural-activity relationships of a variety of phospholipids that are structurally related to PAF (see references 8 and 9 for exhaustive coverage of this subject).

Two important early discoveries in studies of PAF analogs were that only the natural stereoisomer of PAF (the L-form, i.e., the isomer with phosphocholine at the *sn*-3 position), but not the D isomer, exhibited biologic activity (46, 47) and that a nonmetabolizable 2 carbon grouping (ethoxy) substituted at the *sn*-2 position for the acetyl group possessed small but significant PAF activity (46). Other studies (48) with analogs of PAF that lack oxygen at the *sn*-2 position also indicate that transfer of the acetyl moiety from PAF to some other compound is not essential for expressing the biologic activity. Moreover, 1-alkyl-2-*N*-methylcarbamyl-GPC (another nonmetabolizable analog of PAF) exhibited a biologic potency similar to that of PAF (49). The stereospecificity requirement originally described by Wykle *et al.* (46) suggested that a receptor-mediated mechanism was involved in PAF responses and the results obtained with the nonmetabolizable analogs (48, 49) indicated that hydrolysis or transfer of the *sn*-2 acetate to some other molecule was not essential for the biologic responses.

PAF with a 16:0 alkyl chain at the *sn*-1 position is more active than PAF with an 18:0 alkyl chain (50, 51) and species containing unsaturated alkyl moieties have been reported to be more potent than saturated species in some systems (52, 53) but not all (51). Also a noteworthy observation is that the magnitude of responses for any given PAF analog was similar when assayed for either hypotensive activity in rats (a species whose platelets are refractive to aggregation) or serotonin release from rabbit platelets (54). Thus, in studies of the biologic activities of PAF analogs, it seems that a single biologic test system can generally be used to predict the relative potency of an analog's ability to

Table I. Relative Biologic Activity of Various Phospholipid Analogs of PAF[a]

Highest activity (10^{-11}–10^{-10} *M*)
PAF (1-alkyl-2-acetyl-GPC)
1-alkyl-2-propionyl-GPC
1-alkyl-2-*N*-methylcarbamyl-GPC
Intermediate activity ($\approx 10^{-8}$ *M*)
1-alkyl-2-acetyl-*sn*-dimethylethanolamine
1-alkyl-2-acetyl-*sn*-monomethylethanolamine
1-alkyl-2-acetyl-*sn*-glycerol
Low activity (10^{-8}–10^{-7} *M*)
1-alkyl-2-ethoxy-GPC
1-alkyl-2-butyryl-GPC
1-alkyl-2-hexanoyl-GPC
1-alkyl-2-*N*-acetyl-GPC
Inactive ($<10^{-7}$ *M*)
1-acyl-2-acetyl-GPC
1,2-diacetyl-GPC
2-acetyl-3-alkyl-*sn*-glycero-1-phosphocholine
1-alkyl-2-octadecenoyl-GPC
1-alkyl-2-lyso-GPC
1-alkyl-2-methoxy-GPC
1-alkyl-2-benzoxy-GPC
1-alkyl-2-*N*-formyl-GPC
1-alkyl-2-*N*-hexadecanoyl-GPC
1-alkyl-2-dimethyl-GPC
1-alkyl-2-propyl-GPC
1-alkyl-2-isopropyl-GPC
1-alkyl-2-isobutyryl-GPC
1-alkyl-2-acetyl-GPE
1-alkyl-2-acetyl-*sn*-glycero-3-phosphoethanol
1-alkyl-2-acetyl-*sn*-glycero-3-P

[a] These groupings of activities are based mainly on responses obtained with platelet aggregation of rabbit platelets and/or hypotensive effects in rats. Significant differences in absolute activities are encountered with different cell types and animal species; nevertheless, the order for activities of the listed phospholipids in relation to PAF potency would be similar.

exert a biologic response, although an exception has been noted (55).

An important goal in the studies of PAF analogs has been to develop one that possesses only the hypotensive property, without affecting platelet and neutrophil functions. An example of progress in this direction has appeared in a report by Ohno *et al.* (55) who synthesized a PAF agonist [1(*S*)-methyl PAF] that is approximately 2500 times more potent than PAF as a hypotensive agent, but has weaker effects on platelets and neutrophils than PAF does. Inhibitors are also being sought for specific enzymatic steps involved in PAF biosynthesis involved in allergic and inflammatory responses. Although inhibitors of the acetyltransferase in the remodeling pathway of PAF biosynthesis have been described (56), they are not highly specific. Nevertheless, the fact that the enzyme activities in the *de novo* (physiologic) and remodeling (inflammatory) pathways for PAF synthesis are different (see Part VA) offer great promise for the development of enzymatic inhibitors. Thus, research efforts continue to flourish in search for new types of PAF analogs that might have potentially useful clinical applications; except for the PAF antagonists described in Part IV, progress in the development of enzyme inhibitors has been slow.

IV. Antagonists and Receptors of PAF

During the past several years, a variety of PAF receptor antagonists have been obtained via chemical synthesis and isolation from natural products. The subject of antagonists is beyond the scope of this minireview and the reader is referred to two recent comprehensive summary articles on PAF antagonists (4, 56). Chemical structures of antagonists such as CV-3988 are similar to PAF, whereas others such as kadsurenone, WEB 2086, and BN 52021 are structurally dissimilar from PAF. Computerized studies (57) of the molecular spacing arrangements of the chemical structures of different antagonists have revealed that all share common spatial features that are essential to accommodate their interaction with the putative PAF receptor.

A variety of binding experiments with [^{3}H]PAF have shown that high affinity binding sites exist in platelets from humans (58–60), rabbits (61–63), dogs (64), human neutrophils (65, 66), smooth muscle cells (62), P388D$_1$ murine macrophages (67), and human lung tissue (68). Characteristics of binding sites for PAF on the surface of platelets isolated from humans, rabbits, and rats have also been compared in the same laboratory (69). Binding studies carried out at low temperatures have shown a very low number of specific PAF-binding sites for rabbit platelets (150–400 receptors per cell) (61, 62), human platelets (250 receptors per cell) (59), and human neutrophils (1100 receptors per cell) (64). The number of receptors for human platelets at the time of maximal aggregation was determined to be approximately 125 receptors per cell (60).

Specific binding proteins for PAF have also been isolated from human platelets (70, 71). Also of interest is that treatment of neutrophils (72) or platelets (73) from humans with subaggregating concentrations of PAF causes a desensitization of these cells to subsequent levels of PAF that would normally induce their aggregation.

V. Metabolism of PAF and Related Lipids

A. Enzymes for PAF Biosynthesis. PAF can be enzymatically synthesized by either a remodeling or a *de novo* pathway (Fig. 2); each has a distinctively different set of characteristics (Table II). The remodeling route involves the replacement of a long chain acyl group with an acetate moiety derived from acetyl-CoA in a reaction sequence that converts 1-alkyl-2-acyl-GPC to PAF via a lyso intermediate (1-alkyl-2-lyso-GPC, i.e., lyso-PAF); a phospholipase A_2 and an acetyl-CoA acetyltransferase activity are jointly responsible for the transformation of the inactive storage form of the ether lipid to the biologically active molecule (74). The acetyltransferase in the remodeling pathway has been investigated extensively (6, 9), whereas little is known about the precise nature of the phospholipase A_2 in this route.

Alternately, PAF can be produced by a *de novo* route beginning with 1-alkyl-2-lyso-*sn*-glycero-3-P (an alkyl analog of lysophosphatidic acid), an important branch point intermediate in the ether lipid biosynthetic pathway. This intermediate is derived from acyl-dihydroxyacetone-P (DHAP) and a long chain fatty alcohol in a stepwise series of reactions catalyzed by alkyl-DHAP synthase (Fig. 2; Rx I) and NADPH:alkyl-DHAP oxidoreductase (Fig. 2; Rx II). As seen in Figure 2, 1-alkyl-2-lyso-*sn*-glycero-3-P occupies a pivotal position in ether lipid biosynthesis since it can be either acylated by an acyl-CoA (long chain) acyltransferase (Fig. 2; Rx V) or acetylated by an acetyl-CoA acetyltransferase (Fig. 2; Rx XII) to form the corresponding alkyl ether analogs of phosphatidic acid. The *sn*-2 acetyl and *sn*-2 long chain acyl forms of the alkyl type of phosphatidic acid can then be converted to 1-alkyl-2-acetyl-GPC (PAF) or 1-alkyl-2-acyl-GPC (substrate for remodeling route), respectively, in reaction steps catalyzed by a phosphohydrolase (Fig. 2; Rxs VI and XIII) and two different cholinephosphotransferases (Fig. 2; Rxs VII and XIV). Although some reactions in the pathway depicted in Figure 2 are analogous in nature, the pairs of acetyltransferases (75), phosphohydrolases (76), or cholinephosphotransferases (26, 77) appear to be distinctly different enzymes.

In addition to the production of PAF via the *de novo* pathway, this route can also generate alkylacetylacylglycerols (78), a neutral lipid analog of triglycerides. The acyl-CoA:1-alkyl-2-acetyl-*sn*-glycerol acyltransferase that catalyzes this reaction (Fig. 2, Rx XV) also differs in its properties from the analogous acyl-

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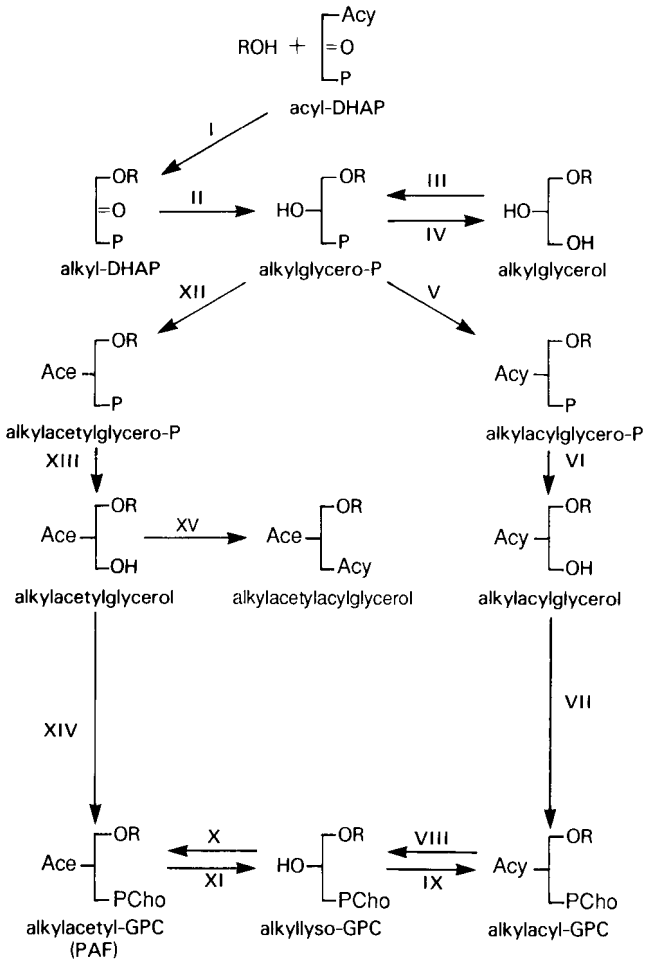

Figure 2. General pathways for the metabolism of the alkyl ether lipids and PAF. The Roman numerals refer to the following enzymes: (I) alkyl-DHAP synthase; (II) NADPH:alkyl-DHAP oxidoreductase; (III) ATP:1-alkyl-*sn*-glycerol-3-P phosphotransferase; (IV) 1-alkyl-2-lyso-*sn*-glycero-3-P phosphohydrolase; (V) acyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-P acyltransferase; (VI) 1-alkyl-2-acyl-*sn*-glycero-3-P phosphohydrolase; (VII) CDP-choline:1-alkyl-2-acyl-*sn*-glycerol DTT-*sensitive* cholinephosphotransferase; (VIII) phospholipase A_2; (IX) phosphatidylcholine:1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine polyenoic-specific transacylase (CoA-independent); (X) acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine acetyltransferase; (XI) 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase; (XII) acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-P acetyltransferase; (XIII) 1-alkyl-2-acetyl-*sn*-glyero-3-P phosphohydrolase; and (XIV) CDP-choline:1-alkyl-2-acetyl-*sn*-glyercol DTT-*insensitive* cholinephosphotransferase; and (XV) acyl-CoA:1-alkyl-2-acetyl-*sn*-glyercol acyltransferase. Ace, acetyl; Acy, acyl; Cho, choline; GPC, *sn*-glycero-3-phosphocholine.

transferase that acylates long chain diacylglycerols (78). Although the physiologic role of alkylacetylacylglycerols is unknown, they could be an important precursor source of alkylacetylglycerols in some cells.

As mentioned earlier (Part II), significant quantities of the acyl analog of PAF have been reported to be formed in various cells. The occurrence of the acyl analog of PAF can be explained on the basis of the substrate specificity of the enzymes responsible for PAF

Table II. General Features Characteristic of the *de novo* and Remodeling Pathways for PAF Biosynthesis

De novo route from 1-alkyl-2-lyso-*sn*-glycero-3-P via alkylacetylglycerols
Maintains physiologic levels of PAF
Virtually the exclusive pathway in kidney
Does not result in formation of eicosanoid mediators
Highest specific activities in resting cells
Not stimulated by inflammatory agents
Remodeling route from alkylacylglycerophosphocholines via lyso-PAF
Route involved in inflammatory responses, i.e., stimulated by inflammatory agents
Eicosanoid mediators formed by simultaneous release of 20:4 during PAF formation
Low activities in resting cells

biosynthesis. The acetyltransferases in both the *de novo* (unpublished data) and remodeling (25, 79) pathways effectively utilize acyllysoglycero-P and acyllyso-GPC, respectively. Similarly, the dithiothreitol (DTT)-insensitive cholinephosphotransferases in the *de novo* route (77) can transfer the phosphocholine moiety to acylacetylglycerol equally as well as to alkylacetylglycerol and the phosphohydrolase that hydrolyzes the phosphate group from alkylacetylglycero-P can also utilize acylacetylglycero-P (76).

The ethanolamine plasmalogen analog of PAF can also be produced as seen in experiments when human neutrophils, stimulated with calcium ionophore A23187, are incubated with [^{3}H]acetate (80). In the same studies, sonicates from the stimulated cells were shown to possess an acetyltransferase that could form 1-alk-1′-enyl-2-[^{3}H]acetyl-*sn*-glycero-3-phosphoethanolamine from [^{3}H]acetate and 1-alk-1′-enyl-2-lyso-glycero-3-phosphoethanolamine. The significance of the ethanolamine plasmalogen analog of PAF in neutrophils is not known, but the choline plasmalogen analog can elicit platelet aggregation, which can be completely abolished by PAF antagonists (81).

B. Enzymatic Inactivation of PAF. The primary route for the intracellular inactivation of PAF is catalyzed by a cytosolic acetylhydrolase (27) that hydrolyzes the acetate moiety to produce lyso-PAF. This lyso intermediate is either converted to 1-alkyl-2-acyl-GPC by a polyenoic-specific transacylase or further catabolized as described below. Acetylhydrolase activity also occurs in the plasma (82–85) and it has recently been purified from human plasma to near homogeneity (84). Interestingly, the plasma acetylhydrolase only appears to be active when associated with low density lipoproteins (contains about 70% of the total plasma acetylhydrolase activity); the remaining acetylhydrolase in plasma is bound to the high density lipoprotein fraction in an inactive form (85).

The extracellular acetylhydrolase appears to have the same catalytic properties as the intracellular enzyme

(82), and it is thought that the serum acetylhydrolase probably originates from the intracellular enzyme pool since the extracellular activity is resistant to proteolytic hydrolysis and has a somewhat higher molecular weight than the intracellular acetylhydrolase (82). These results are consistent with the idea that processing of the intracellular acetylhydrolase for secretion from cells could involve a glycosylation step as is common for a number of other secretory proteins.

Once the acetate is hydrolyzed from PAF by the intracellular acetylhydrolase, a microsomal transacylase rapidly acylates the lyso-PAF intermediate (86–95). The transacylase is highly specific for arachidonic acid and other polyenoic acids that appear to originate from phosphatidylcholine (86, 91, 92, 94). The transacylation reaction accounts for enrichment of arachidonate and other polyunsaturates in the ether-linked phospholipids; in many cells 1-acyl-, 1-alkyl-, and 1-alk-1-enyl-lysophospholipids can serve as acceptor molecules in the transacylase reaction (86, 90, 92).

Direct inactivation of PAF by a phospholipase C has also been proposed (96, 97), but the hydrolytic rates are considerably lower than the acetylhydrolase and, therefore, the relevance of phospholipase C in PAF catabolism is questionable. Other enzymes that can play an important role in PAF catabolism are the Pte·H_4-dependent alkyl cleavage enzyme (98) and lysophospholipase D (99–102). These enzyme activities are specific for lipids with ether linkages and both can utilize lyso-PAF as a substrate.

C. Regulatory Controls. The regulation of PAF metabolism is still poorly understood. Based on the characteristics of enzymes involved in the biosynthesis of PAF, it is clear that crucial signals for turning on and off the production of PAF are metal ions, thiol compounds, fatty acids, pH, compartmentalization, and the phosphorylation and dephosphorylation of enzymes. Divalent cations are especially important in regulating PAF metabolism. Calcium not only inhibits the acetyltransferase (75), phosphohydrolase (76), and the DTT-insensitive cholinephosphostransferase (77) in the *de novo* pathway of PAF biosynthesis, but it also can inhibit the deacetylation and transcylation steps in the remodeling pathway both *in vivo* and *in vitro* (103). On the other hand, calcium is essential for PAF production via the remodeling pathway (104–106). Calcium is also required for lysophospholipase D activity in rabbit tissues (102), but has no effect in rat tissues where the metal requirement is known to be Mg^{2+} (99–101).

It has been established that the acetyltransferase activity in the remodeling pathway is regulated by an activation/inactivation mechanism involving phosphorylation/dephosphorylation (107–112). Some experimental evidence suggests that a cAMP-dependent kinase (107, 108) is responsible and that the ^{32}P from [$\gamma^{32}P$]ATP is incorporated into the serine residue of the enzyme (109). However, the calcium/calmodulin-dependent protein kinase is thought to be responsible for phosphorylating the acetyltransferase in microsomes of guinea pig parotid glands (108). Even though the cAMP-dependent protein kinase can activate the enzyme in the microsomal preparations from this gland, experiments with intact cells showed that β-agonists, which are known to activate the cAMP-dependent kinase, did not affect the cellular acetyltransferase activity (108).

Whereas protein kinase C is not capable of activating the acetyltransferase, it is thought that it might be involved in the activation of the phospholipase A_2 that forms the lyso intermediate in the remodeling pathway through its action on alkylacylglycerophosphocholines (113). This conclusion is based on the fact that inhibitors of protein kinase C inhibit the stimulation of PAF biosynthesis by ionophore A23187 and activation of protein kinase C with phorbol-12-myristate-13-acetate reverses the inhibition.

Arachidonic acid also appears to play an important role in regulating the remodeling pathway of PAF biosynthesis. Stimulated cells depleted of arachidonate are unable to produce PAF (114) and it is known that arachidonic acid supplements (114) and 5-lipoxygenase products enhance the formation of PAF via the remodeling route, presumably by stimulation of phospholipase A_2 activity (115).

Rate limiting steps in the de novo synthesis of PAF appear to be acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-P acetyltransferase (75) and the cytidylyltransferase that forms CDP-choline (116). Factors that affect either enzyme activity will modulate the amount of PAF formed *de novo*. For example, oleic acid is known to activate cytidylyltransferase by causing the translocation of this enzyme from the cytosol to cellular membranes where it exists in an active form; after treatment of Ehrlich ascites cells (a cell that normally produces only small amounts of PAF) with oleic acid, PAF synthesis from alkylacetylglycerols is stimulated about 10-fold over controls (116). Phorbol-12-myristate-13-acetate, which is known to stimulate cytidylyltransferase activity (117), also enhances PAF biosynthesis via the *de novo* pathway (118). Neurotransmitters have been shown to stimulate *de novo* PAF synthesis in chick retinas (119), but the mechanism for this stimulation is unknown at present. Needless to say, the regulation of the *de novo* pathway for PAF biosynthesis must be under very stringent control in view of the potential damaging and lethal effects of high levels of PAF.

Endogenous lipid inhibitors of PAF actions (aggregation of rabbit platelets) have been reported to occur in the liver (120) and uterus (121) of rats. The inhibitors isolated from the uterus were identified as acyllyso- and alkyllyso-GPC containing mixed aliphatic chains and a

mixture of *N*-acyl-sphing-4-enyl-phosphocholine (121). The physiologic significance of these endogenous lipid inhibitors of PAF responses is still poorly understood. Nonetheless, the fact that such inhibitors can co-migrate with PAF in chromatography systems (120) can add to difficulties in the interpretation of negative biologic responses with naturally isolated PAF preparations.

VI. Biochemical Mode of Action of PAF

As an autocoid, PAF interacts with specific receptors of certain cells that initiate events associated with the signal transduction process. The exact sequence of events and biochemical mechanism in this process are still unknown, but experimental evidence suggests that the activated PAF receptor might interact with a GTP-binding protein (122–126) in the membrane.

Human platelet membranes exposed to either cholera or pertussis toxin, agents known to inhibit the GTPase activities in N_s and N_i, respectively, have little effect on the GTPase activity stimulated by PAF (126). These results indicate that the effects of PAF receptor interaction in human platelets are via a GTP regulatory protein that differs from N_s and N_i. Nevertheless, pretreatment of human neutrophils with pertussis toxin inhibited PAF-induced chemotaxis, which suggests that the GTP regulatory protein N_i could play a role in these cells (122).

Linkage between the PAF receptor and adenylate cyclase has been proposed based on inhibitory and potentiating effects of sodium and magnesium, respectively, on PAF binding (123). Moreover, PAF is known to inhibit adenylate cyclase in platelets (125, 127).

Also of interest with regard to the mode of PAF action is that serine protease inhibitors can inhibit PAF-induced responses (platelet aggregation and secretion) after PAF is bound to its receptor (128). Sugatani *et al.* (128) concluded from these results that a chymotryptic type serine protease might contribute to platelet stimulation induced by PAF. Zinc ions also appear to play a role in the activation of platelets (129).

The significant quantities of PAF found in intracellular compartments has raised questions about its possible function within cells. Henson (130) has discussed some of the possible implications of the dual roles of PAF as both an extracellular and intracellular mediator. However, at present, the significance of PAF in intracellular processes is not established.

VII. PAF in Health and Disease

PAF is thought to be an important signal in reproduction, fetal development, and the initiation of parturition (4, 131). O'Neill (132) has reported that mouse embryos produce PAF and subsequent experiments have shown the formation of PAF *in vitro* correlates with the viability and pregnancy potential of the embryo (133). These data are consistent with the concept that PAF is essential for implantation of the fertilized egg in the uterus, which is further emphasized by the fact implantation of mouse embryos is inhibited following injections of the specific PAF antogonist, SRI-63-441 (134). The observation that PAF appears in amniotic fluid in association with labor (135) prompted Johnston *et al.* (131) to conduct a number of exciting studies that demonstrate PAF is involved in fetal development and parturition (136). An excellent and comprehensive review of this interesting area has been written by Johnston *et al.* (131).

The hypotensive activity of PAF and its formation in the kidney almost exclusively by the *de novo* biosynthetic pathway (77) has implied that PAF production by kidney cells could be a physiologic factor in contributing to the control of blood pressure. Higher levels of serum acetylhydrolase found in spontaneous hypertensive rats (82) and white males with hypertension (137) support this concept. The lower levels of circulating PAF in blood during renal one-clip kidney induced hypertension and the increased levels of PAF in blood after reversal of the hypertension by removal of the arterial clip further suggest that PAF is involved in maintaining normal blood pressure (138).

In addition to hypertension, PAF has been implicated as a factor in a variety of diseases. Some disorders attributed at least in part to PAF include: thrombosis, acute inflammation, asthma, systemic anaphylaxia, cardiac anaphylaxis, endotoxic and IgG-induced shock, gastrointestinal ulceration, inflammatory and allergic skin diseases (e.g., psoriasis), and retinal and corneal diseases. The vast literature related to the subject of PAF and disease has recently been discussed in a review by Braquet *et al.* (4).

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