REVIEW ARTICLE

Platelet-activating factor: receptors and signal transduction

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INTRODUCTION

In 1970, during a study of immunological mechanisms involving histamine and serotonin release from platelets in immunized rabbits, Henson [1] proposed that 'a soluble factor' was released from leukocytes which stimulated platelets to release vasoactive amines. This observation was confirmed independently by Siraganian and Osler [2] in 1971. In 1972, Benveniste, Henson and Cochrane [3] demonstrated that the antibody involved in the immunological reaction described above was an IgE class antibody and coined the term 'platelet-activating factor (PAF)' for the soluble factor released from basophils following IgE stimulation. Several reports followed describing the lipid character of PAF [4–6]. However, it was not until 1979 that Demopoulos, Pinckard and Hanahan [7] demonstrated that a semisynthetic phosphoacylglycerol, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (AGEPC), had physiochemical as well as biological properties (i.e. aggregation of platelets and secretion of serotonin) indistinguishable from those of naturally-generated PAF [6]. At the same time, Blank et al. [8] reported independently the preparation of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine from choline plasmalogens isolated from bovine heart by using the same semisynthetic approach used by Demopoulos et al. [7]. The compound synthesized by Blank et al. possessed profound antihypertensive properties in the rat. Shortly after these events, Benveniste et al. [9] reported the chemical preparation of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, which had similar biological properties to those of naturally-occuring PAF.

After these initial attempts to characterize the chemical structure of PAF, Hanahan et al. [10] isolated PAF from sensitized rabbit basophils and characterized the chemical structure of the naturally-occuring substance by using gas–liquid chromatography and mass spectral analysis and demonstrated that naturally-occuring PAF was indeed AGEPC. The structure of this novel ether lipid is shown in Figure 1.

Several important features of this phosphoacylglyerol mediator were revealed through evaluation of the functional capacities of structural analogues of PAF (reviewed in [11,12]). PAF has an *O*-alkyl ether residue at the *sn*-1 position and a short acyl chain, i.e. an acetyl moiety, at the *sn*-2 position. The *sn*-3 position is occupied by the polar head group *O*-phosphocholine. Several modifications, such as (a) acyl analogues at the *sn*-1 position, (b) different chain lengths of the ester group beyond three carbon atoms or a hydroxyl group at the *sn*-2 position, and (c) ethanolamine substituted for choline at the *sn*-3 position, greatly diminish or even abolish biological activity.

During the past decade, the elucidation of the biological properties of PAF has indicated that this molecule is involved extensively in intercellular signalling in a variety of pathophysiological situations. Numerous cell types and tissues have been shown to synthesize and release PAF upon stimulation and at the same time to exhibit biological responses to this compound (Table 1). There are two metabolic pathways involved in the biosynthesis of PAF, the remodelling and the *de novo* pathways. The details of the synthetic and degradative pathways for PAF

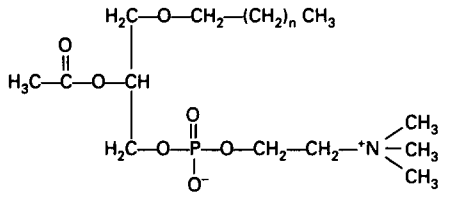

Figure 1 Chemical structure of PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine); *n* = 14–16

Table 1 Biosynthesis of PAF in various cells in response to different stimuli

Cell and tissue	Stimuli	Reference
Endothelial cells	Thrombin	152,153
	Bradykinin, A23187	154
	Tumour necrosis factor	155,156
	Interleukin 1α	156
	Leukotrienes C_4 and D_4	157
	Interleukin 1	158
	Histamine, bradykinin, ATP	159
	A23187	160
Neutrophils		
Human	Zymosan	161
	A23187	162–165
	fMet-Leu-Phe	166
	Zymosan and A23187	167
Rat	A23187	168
	Tumour necrosis factor	155
Rabbit	A23187	169,170
Platelets	Thrombin	171
Macrophages	Zymosan, A23187	172,173
	A23187	174
	Zymosan	175–178
	Tumour necrosis factor	155
HL-60 cells	A23187	179
Rat Kupffer cells	A23187	142
Exocrine cells	Carbachol	180
Eosinophils	A23187	165
Rat kidney cells	A23187	181

Abbreviations used: PAF, platelet-activating factor; AGEPC, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; alkylacylGPC, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine; lysoPAF, 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine; LPS, lipopolysaccharide; PG, prostaglandin; TX, thromboxane; LT, leukotriene; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; PMA, phorbol 12-myristate 13-acetate; GTPγS, guanosine 5-[3-*O*-thio]triphosphate; DAG, diacylglycerol; ROI, reactive oxygen intermediates.

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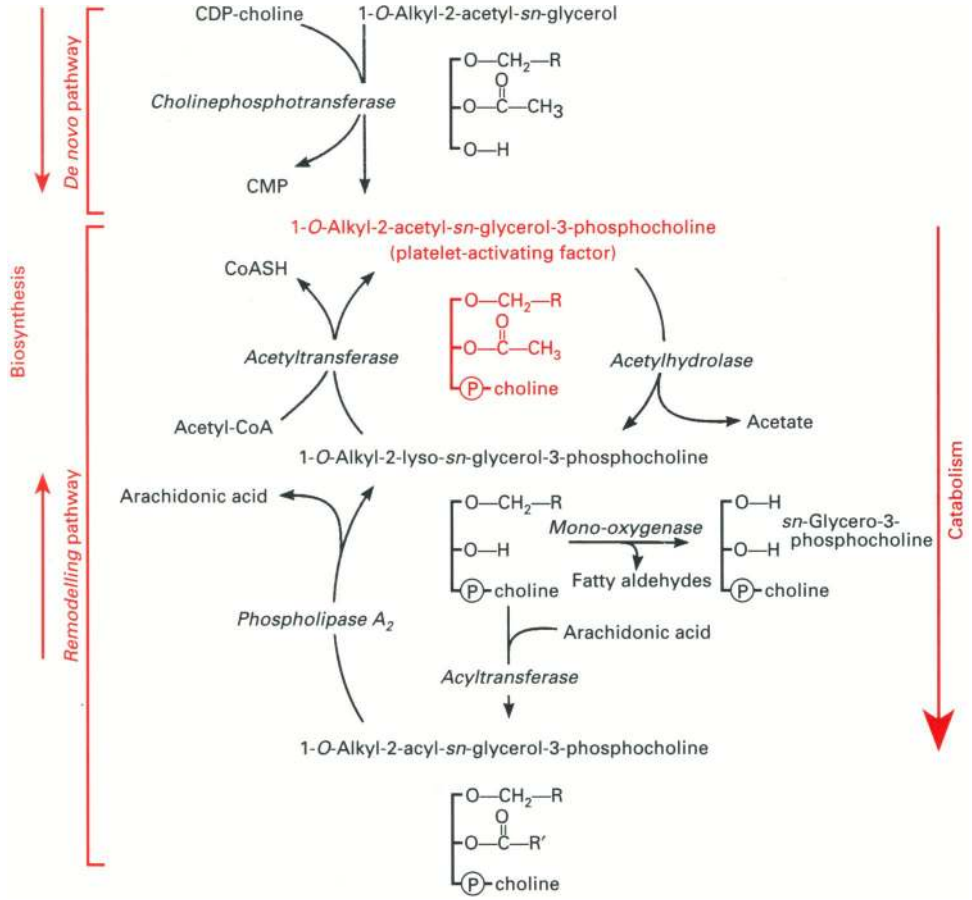

Figure 2 Metabolic pathways of PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine)

Table 2 Physiological and pathophysiological effects of PAF

Platelet aggregation and secretion, thrombosis
Stimulation of neutrophils and macrophages
Acute inflammation
Asthma and systemic anaphylaxis
Endotoxin and immune factor-induced shocks
Gastrointestinal ulceration
Glycogenolysis and increased portal pressure
Pancreatitis
Cardiac anaphylaxis (negative inotropic effect and increased heart beating rate)
Pregnancy and ovoimplantation
Ovulation
Acute lung injury

are summarized in Figure 2. Ligand binding studies indicate that specific PAF receptors can be identified in various cells and tissues (reviewed in [13]). Such a wide distribution of PAF receptors suggests that PAF must be an important mediator involved in cellular responses to trauma. Several studies have indicated that the PAF receptor is under stringent regulation as it functions in the cellular signalling mechanisms in which it plays a role. Successful cloning of the PAF receptor gene from guinea pig lung and several other types of cells represents a significant breakthrough which will allow further detailed examination of the PAF receptor and the attendant signalling mechanisms which depend upon its participation [14–16]. Following the activation of specific PAF receptors, diverse biochemical effects are elicited, including activation of phospholipases C and A_2 leading to hydrolysis of phosphoinositide and release of arachidonic acid, respectively, an increased cytosolic calcium concentration, activation of protein kinase C, protein tyrosine phosphorylation, and proto-oncogene expression. These biochemical studies provide insight into the complex intracellular signalling mechanisms of PAF. There is substantial evidence that PAF plays an important role in various physiological and pathophysiological episodes. Table 2 summarizes some of these situations. Several comprehensive reviews have focused on this rapidly developing area [11,12,17–19]. This article will discuss the characterization and regulation of the PAF receptor and PAF receptor-mediated transmembrane signalling mechanisms.

PAF RECEPTORS

Identification and characterization

Specific receptors for PAF have been identified in numerous tissues and cells (Table 3). The first binding experiment utilizing [^{3}H]PAF was conducted in human platelets in 1982 [20]. Using [^{3}H]PAF (a mixture of 1-*O*-hexadecyl- and 1-*O*-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) in the absence or presence of excess unlabelled PAF at 20 °C , two distinct types of binding sites were revealed. One binding site for PAF on platelets exhibited a high affinity with a K_d value of 37 ± 13 nM and had a low capacity of 1399 ± 498 sites/platelet. The other binding site possessed nearly infinite binding capacity with a low affinity for

Table 3 Specific PAF receptors in various tissues and cells

Cell and tissue	K_d (nM)	$B_{max.}$ (sites/cell, or as indicated)	Reference
Human platelet	37 ± 13	1399 ± 498	20
Human platelet	1.58 ± 0.36	1983 ± 391	22
Human platelet	0.05	242 ± 64	21
Rabbit platelet	0.5	400	66
Rabbit platelet membrane	1.36	150–300	25
Rat platelet	No specific PAF binding		22
Neutrophil	0.11 ± 0.02	5×10^6	26
Neutrophil	45	2.8×10^4	27
Neutrophil membrane	0.2	1100	28
Macrophage	0.08	7872	29
	0.25	117 fmol/mg of protein	69
Mononuclear leukocyte	5.7	1.11×10^4	31
Lung membrane	0.49	140 fmol/mg of protein	182
Liver membrane	0.5 ± 0.14	140 ± 18 fmol/mg of protein	183
Gerbil brain membrane	3.66 ± 0.92 (high)	0.83 pmol/mg of protein	40
	20.4 ± 0.5 (low)	1.1 pmol/mg of protein	40
Synaptic plasma membrane	0.023 (high)	8.75 fmol/mg of protein	41
	25 (low)	0.96 pmol/mg of protein	41
Rat retina	2.9	0.85 pmol/mg of protein	42
Eosinophil	1.6	3.5×10^4	32
Kupffer cells	0.12–0.45	1.06×10^4	33

PAF. The high-affinity binding sites were responsible for PAF-elicited platelet aggregation. By comparing the capacity of several analogues of PAF (i.e. lysoPAF and 1-hexadecyl-2-benzoyl-*sn*-glycero-3-phosphocholine) to inhibit the specific binding of [^{3}H]PAF and to induce platelet aggregation, it was demonstrated that several features of the PAF structure described above (an ether linkage at the *sn*-1 position, an acetyl moiety at the *sn*-2 position, and a polar head group containing choline at the *sn*-3 position) were critical both for the specific PAF binding to human platelets and for the initiation of platelet aggregation [20]. Similar to human platelets [20,21], rabbit platelets possess high-affinity binding sites for PAF of K_d 0.9 ± 0.5 nM [22], while rat platelets show only non-specific binding, explaining perhaps the observation that the functional responses of the rat platelet are insensitive to PAF [23]. Platelets from patients with septicaemia exhibit fewer specific [^{3}H]PAF binding sites compared with platelets from normal humans or from patients with respiratory or cardiovascular disturbances [24]. In addition, platelets from patients with sepsis contained significant amounts of PAF, whereas this mediator could not be found in platelets from patients with either respiratory or cardiovascular disturbances with negative blood cultures, or from normal individuals. The observation that a large amount of PAF is associated with one of its target cells under certain pathophysiological conditions such as sepsis may provide the rationale for using PAF antagonists in patients with severe shock or multiple organ dysfunction.

Specific binding sites for PAF are present on smooth muscle cells [25], neutrophils [26–28], macrophages [29,30], mononuclear leukocytes [31], eosinophils [32], and Kupffer cells [33]. In neutrophils, there exist two classes of binding sites [26–28,34], high- and low-affinity (Table 3). The high-affinity binding sites are believed to mediate PAF-induced cellular responses. The typical number of PAF receptors found on neutrophils ranges from several hundred to thousands. Both murine [29,30] and rat [33] macrophages possess high-affinity receptors for PAF. PAF receptors following ligand binding mediate several biochemical activities including phosphoinositide and arachidonic acid metabolism, intracellular calcium changes, and protein phosphorylation.

A problem with some of the early studies of PAF binding to its receptors in intact cells was that data on [^{3}H]PAF metabolism under the binding conditions were not presented. The lack of information on PAF metabolism renders the binding data difficult to interpret since it is known that many cells actively metabolize PAF [28,35–37]. O'Flaherty et al. [28] characterized the binding and metabolism of [^{3}H]PAF by human neutrophils and showed that neutrophils rapidly metabolize [^{3}H]PAF to its alkylacyl derivative at 37 °C. Subcellular fractionation of cells pretreated with radiolabelled PAF on Percoll gradients revealed that most [^{3}H]PAF associated with alkaline phosphatase-rich membranes was converted rapidly to alkylacyl-GPC and was transferred slowly to specific intracellular granules. In contrast, human neutrophils did not metabolize [^{3}H]PAF at 4 °C, but rather accumulated PAF in plasma membrane subfractions. Under non-metabolizing conditions, [^{3}H]PAF binding experiments indicated that human neutrophil plasma membranes possess two classes of binding sites, high-affinity and low-affinity, with K_d values of 0.2 nM and 500 nM, respectively. The potency of several structural analogues in inhibiting binding of [^{3}H]PAF to membranes correlated closely with their respective potency in stimulating degranulation responses.

A possible physiological and pathophysiological role for PAF in tissue or cells of the central nervous system was first suggested by Kornecki and Ehrlich [38] and by Kumar et al. [39]. It was found that incubation of cultured NG108-15 neuroblastoma cells for 3–4 days with low concentrations of PAF caused neuronal differentiation while higher concentrations of PAF were neurotoxic. In addition, PAF caused an almost immediate increase in intracellular free Ca^{2+} in both NG108-15 and PC12 cells. This effect was dependent upon extracellular calcium and was inhibited by the PAF receptor antagonist CV-3988. The functional consequence of the increase in intracellular Ca^{2+} was a secretion of ATP from PC12 cells [38]. PAF caused an accelerated turnover of ^{32}P-labelled phosphoinositides in a synaptosome preparation from rat brain [39]. Most interesting

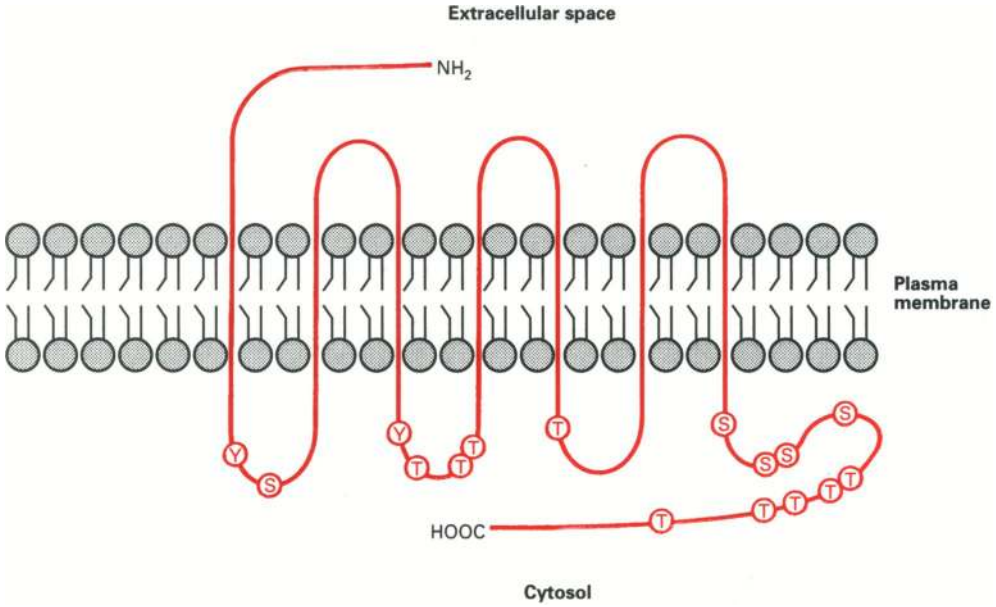

Figure 3 Schematic representation of PAF receptor

Guinea pig lung PAF receptor has 342 amino acids and a molecular mass of 38982. There are seven transmembrane segments. The possible intracellular phosphorylation sites of the PAF receptor [14] are illustrated. S, serine residues; T, threonine residues; Y, tyrosine residues.

was the observation that both electrical and chemical stimulation of either the isolated perfused rat brain or the brain of an intact animal resulted in a substantial increase in PAF levels in the brain [39]. Such observations suggest an important role(s) for PAF in the brain under various physiological and pathophysiological situations. Supporting this suggestion was an investigation conducted by Domingo et al. [40] in which specific [^{3}H]PAF binding sites on membrane preparations from gerbil brain were characterized. Scatchard analysis revealed two apparent populations of binding sites (Table 3). A study of the distribution of [^{3}H]PAF binding revealed that maximal binding was present in the midbrain and hippocampus. Marcheselli et al. [41] found three distinct classes of binding sites for PAF in synaptic plasma membranes and in intracellular membranes of rat cerebral cortex. The significance of intracellular PAF receptors has not been clarified but it has been postulated that intracellular PAF receptors may mediate PAF-induced proto-oncogene expression in this type of tissue [42].

Early attempts to purify and to characterize PAF receptor proteins from different sources, mainly from rabbit and human platelets [43–45] have been unsuccessful. Using photoaffinity labelling techniques, PAF binding proteins in rabbit platelet membranes have been characterized [46]. A photoreactive, radioiodinated derivative of PAF, 1-*O*-(4-azido-2-hydroxy-3-iodobenzamido)undecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine ([^{125}I]AAGP), was synthesized and was used to label specific PAF binding proteins. This derivative of PAF maintained the biological effects of authentic PAF, inducing aggregation of rabbit platelets. Photoaffinity labelling of platelet membranes with [^{125}I]AAGP revealed several ^{125}I-labelled components in SDS/polyacrylamide-gel electrophoresis. One labelled component exhibiting an apparent molecular mass of 52000 was observed consistently and its labelling was inhibited significantly by unlabelled PAF at nanomolar concentrations and by the specific PAF antagonists SRI-63,675 and L652,731, but not by lysoPAF. A significant advance was made in this area when Shimizu and his colleagues cloned a cDNA for a PAF receptor from guinea pig lung [14]. The cloning strategy involved the construction of a cDNA library from size-fractionated poly(A) RNA, the synthesis of a transcript of the cDNA *in vitro* using phage DNA as a template, the expression of the transcript in *Xenopus* oocytes, and the electrophysiological detection of a PAF-induced response in the oocytes. The PAF receptor cDNA analysis indicated that the PAF receptor has 342 amino acids and a molecular mass of 38982 Da. A hydropathy profile analysis suggested the existence of seven transmembrane segments and the cytoplasmic tail of the PAF receptor contained four serine and five threonine residues as possible phosphorylation sites (Figure 3). Subsequently, the PAF receptor was cloned from human leukocytes [15,47] and from HL-60 granulocytes [16]. The cloned PAF receptor from human leukocytes has similar characteristics to that of guinea pig lung with 83 % identity in the amino acid sequence. Using the expressed PAF receptor in *Xenopus laevis* oocytes and COS-7 cells, it was demonstrated that the PAF receptor is linked functionally to phosphoinositide metabolism via a G-protein [15].

Linkage with guanine nucleotide regulatory proteins

Numerous studies have indicated that PAF receptor-induced transmembrane signalling mechanisms involve guanine nucleotide regulatory proteins (G-proteins). Although it is clear that the PAF receptor is coupled to various cellular effector systems such as phospholipase A_2 and phospholipase C through G-proteins, the identities of the G-proteins involved have not been characterized.

The initial observations concerning the involvement of G-proteins in PAF action were made in the early 1980s. It was found that synthetic PAF inhibited adenylate cyclase activity in the particulate fraction of platelets [48,49] and stimulated GTPase activity as well [49–53]. In an effort to elucidate the identity and properties of G-protein(s) involved in the activation of platelets,

Brass et al. [54] examined the relationship between various receptors (including the PAF receptor) and G-proteins in human platelets by comparing the ability of various agonists (e.g. PAF) to stimulate phospholipases (via G_p) and to inhibit adenylate cyclase (via G_i) with their ability to induce structural changes in G_p and G_i which would preclude subsequent [^{32}P]ADP-ribosylation. Using permeabilized platelets and platelet membranes, it was found that thrombin, which elicited responses that were mediated by both G_i and G_p, decreased ^{32}P-radiolabelling of G-proteins by > 90%. PAF and vasopressin, which were found to couple only to G_p to stimulate phosphoinositide breakdown, decreased the radiolabelling by 50%, as did adrenaline, which was coupled functionally only to G_i. On the other hand, an agonist that neither inhibited cyclic AMP formation nor caused pertussis toxin-sensitive phosphoinositide hydrolysis, such as the thromboxane analogue U46619, had no effect on [^{32}P]ADP-ribosylation. The [^{32}P]ADP-ribosylation catalysed by pertussis toxin in permeabilized platelets or platelet membranes labelled a protein(s) (α_{41}) which migrated in SDS/polyacrylamide-gel electrophoresis slightly below rabbit and bovine α_i (M_r 41000). Based on experiments involving proteolytic digestion of the G-protein and subsequent two- dimensional electrophoresis, it was concluded that in platelets a single pertussis toxin-sensitive, α_{41}-containing G-protein was invoved in the regulation of both adenylate cyclase and phospholipase C.

In rabbit platelets, PAF receptor stimulation increased GTPase activity [51,52] and this stimulatory effect of PAF on GTPase activity was attenuated in cells pretreated with phorbol ester, dibutyryl cyclic AMP, and by PAF-induced desensitization [51]. It is likely that both homologous desensitization [55] and dibutyryl cyclic AMP [56] modify PAF receptors causing functional dissociation of PAF receptors from their G-proteins. On the other hand, phorbol ester was found to suppress PAF-mediated signal transduction through modification of the GTP-binding proteins, as phorbol ester was found to abolish PAF-stimulated GTPase activity [51]. This observation suggests that protein kinase C selectively inhibits PAF effects by inactivating a GTP-binding protein coupled with PAF receptors, although activation of protein kinase C also down-regulates surface PAF receptors [57–59].

Although evidence has been presented that GTP-binding protein(s) are involved in signal transduction from PAF receptors to a receptor-stimulated phospholipase A_2, the identity and properties of the G-protein regulating the activity of phospholipase A_2 remain uncertain. Pertussis toxin inhibits PAF-elicited arachidonic acid release, PGE_2 formation, and inositol trisphosphate production, but it does not alter significantly the rise in intracellular calcium [60]. Further information concerning the G-protein regulation of phospholipase A_2 was presented by Nakashima et al. [61] in neutrophils and Kajiyama et al. [62] in rabbit platelets. In neutrophils permeabilized with saponin, the guanine nucleotide analogue guanosine 5-[3-*O*-thio]triphosphate (GTPγS) and NaF, which bypass receptors and directly activate G-proteins, induced the release of [^{3}H]arachidonic acid. The effect of GTPγS was inhibited by pretreatment with pertussis toxin. Similar information was obtained from a human neutrophil homogenate and membrane preparation [63] and in platelets [62]. GTPγS, guanosine 5-[$\beta\gamma$-imido]triphosphate and $NaAlF_4$ all caused a significant release of arachidonic acid in digitonin-permeabilized platelets. The stimulatory effect of the GTP analogues was inhibited by guanosine 5-[2-*O*-thio]diphosphate and by pertussis toxin.

Interestingly, GTP-binding proteins may be involved in PAF-stimulated release of PAF. PAF enhanced the release of newly synthesized PAF in human neutrophils, as assessed by [^{3}H]acetate incorporation into PAF. The non-metabolizable bioactive PAF analogue 1-*O*-hexadecyl-2-(*N*-methyl)-carbamoyloxy-*sn*-glycero-3-phosphocholine [64], but not lyso-PAF, enhanced the release of newly-synthesized PAF. The PAF-stimulated PAF release was inhibited in pertussis toxin-treated neutrophils. The biosynthesis of PAF involves activation of at least two critical enzymes, phospholipase A_2 and the PAF acetyltransferase. The observation that pertussis toxin inhibits the PAF-stimulated synthesis of PAF suggests that a G-protein is likely to be involved in the signal transduction from PAF receptors to the PAF-synthesizing enzyme system.

Regulation

Knowledge of the regulatory factors that affect specific PAF receptors and subsequently control PAF-elicited cellular responses remains in the initial stages of development. Generally, any factor affecting the process of PAF binding to its receptor or subsequent PAF receptor-mediated signal transduction is a likely candidate as a regulator of specific PAF receptors.

It has been found that PAF down-regulates its own receptors in platelets [20,21] and in cultured Kupffer cells [55]. Valone et al. [20] showed that pretreatment of human platelets with PAF led to a decrease in specific [^{3}H]PAF binding. The ligand-mediated loss of specific PAF receptors was very rapid, with only 50% of the specific binding sites remaining after 90 s of incubation at 37 °C. Kloprogge and Akkerman [21] confirmed this finding that desensitized human platelets failed to respond to PAF because of a loss of available binding sites for PAF. However, Chesney et al. [65] reported that pretreatment of human platelets with PAF caused a decrease in the binding affinity of specific PAF receptors rather than the loss of binding sites. In contrast, Homma et al. [66] found, in an investigation of the mechanism for the desentization of PAF responses, that functionally desensitized rabbit platelets did not lose their capacity for specific binding of [^{3}H]PAF. Rather, PAF-treated platelets internalized more [^{3}H]PAF than control platelets. A potential problem with the investigations regarding homologous down-regulation of the PAF receptor was that very little attention was given to the extent to which PAF molecules added during pretreatment associate with the platelet membrane after the pretreatment and any subsequent wash procedure. The presence of residual unlabelled PAF from the pretreatment could decrease subsequent specific [^{3}H]PAF binding. The lack of such information renders data concerning the loss of specific [^{3}H]PAF binding sites difficult to interpret since PAF is very hydrophobic and exhibits high non-specific binding to the lipid bilayer of the plasma membrane. A washing procedure employing a high concentration (1%) of bovine serum albumin can remove PAF efficiently from the outer leaflet of cells [55].

In neutrophils [57,58] and rat Kupffer cells [59] the PAF receptor is modulated by protein kinase C. In neutrophils, specific [^{3}H]PAF receptors were modulated by the protein kinase C activator phorbol ester [57,58], as well as by LTB4 [58]. The regulatory effect of protein kinase C on specific PAF receptors was bidirectional, increasing specific [^{3}H]PAF binding at low concentrations and decreasing binding at high concentrations. The protein kinase C regulation of PAF receptors seems to be cell type-dependent since the protein kinase C activator phorbol 12-myristate 13-acetate (PMA) had no effect on specific binding of PAF in rabbit platelets [51]. Although dibutyryl cyclic AMP was reported to have profound inhibitory effects on PAF-stimulated biological responses such as platelet aggregation and GTPase activity, cyclic AMP analogues at concentrations as

high as 2 mM had little apparent effect on [^{3}H]PAF binding in rabbit platelets [51]. However, in cultured Kupffer cells, prolonged incubation with cyclic AMP analogues or forskolin decreased the surface expression of PAF receptors [56]. In U937 cells, dibutyryl cyclic AMP was found to down-regulate PAF receptor mRNA levels after a 72 h treatment [47]. These observations indicate that cyclic AMP down-regulates surface expression of PAF receptors through a mechanism involving decreased PAF receptor gene expression and/or subsequent receptor protein synthesis.

It is evident that various monovalent and divalent cations exert regulatory effects on PAF receptors. Na^+ specifically inhibited [^{3}H]PAF binding at 0 °C, primarily due to a decrease in the affinity of specific PAF receptors while Li^+ was 25-fold less effective and K^+, Cs^+ and Rb^+ enhanced PAF binding. Mg^{2+}, Ca^{2+} and Mn^{2+} enhanced the specific PAF receptor binding 8–10-fold. Scatchard analysis of the binding data suggested that the Mg^{2+}-induced enhancement of specific PAF binding was attributable to an increase in the affinity of the receptor for PAF and an increase in the available specific PAF binding sites. The specific mechanism involved in these cation effects on PAF receptors has not been elucidated [52]. Zinc ions have been shown to have profound inhibitory effects on PAF-induced activation of rabbit [67] and human [68] platelets, as well as [^{3}H]PAF binding [68]. The decrease in specific [^{3}H]PAF binding caused by zinc resulted primarily from a decreased affinity of the specific binding sites rather than a reduced number of binding sites. Compared with Zn^{2+}, Cd^{2+} and Cu^{2+} had much weaker inhibitory effects on specific PAF binding [68].

Recent studies have demonstrated that bacterial lipopolysaccharide (LPS) induces an increase in the surface expression of PAF receptors in macrophages [69]. Consequently, the PAF-induced increase in intracellular Ca^{2+} is enhanced [69,70]. The receptor regulatory effect of LPS is time-dependent with a maximal effect (150–200%) observed within 5 h and 8 h. Cycloheximide and actinomycin D can abolish the effect of LPS, suggesting the involvement of enhanced receptor protein synthesis and mRNA production in this event [69]. The alteration of PAF receptor expression in response to LPS treatment may represent one of the mechanisms for LPS priming of PAF-induced responses such as prostaglandin E_2 production [71].

PAF-MEDIATED BIOCHEMICAL EFFECTS

Arachidonic acid metabolism

During the past decade, a vast literature has been elaborated to define the relationship between PAF action and arachidonic acid metabolism. Various types of cells release arachidonic acid when PAF is synthesized as well as in response to PAF stimulation (Figures 2 and 4). Moreover, it is known that both PAF and arachidonic acid can be released from a common precursor, 1-*O*-alkyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (Figure 2).

Numerous types of cells and tissues release arachidonic acid and its metabolites in response to PAF stimulation. Responsive cells include platelets [72,73], neutrophils [63,74–76], eosinophils [77,78], macrophages [33,56,59,79–81], smooth muscle cells [82], epithelial cells [83], and the perfused heart [84] and liver [85], to name a few. The released arachidonic acid and its metabolites in response to PAF are believed to play an important role in various physiological and pathophysiological processes.

In an *in vivo* study, McManus et al. [73] found that intravenous antigen challenge of IgE-sensitized rabbits caused a significant elevation of plasma TXB_2. Simultaneously, basopenia, thrombocytopenia and neutropenia occurred. The cyclooxygenase inhibitor aspirin, when given to rabbits 18 h before antigen challenge, did not prevent the development of thrombocytopenia, neutropenia, basopenia, or the release of platelet factor 4, but reduced significantly the release of TXB_2 and resulted in mortality in IgE-sensitized rabbits. Based on these observations and data from *in vitro* experiments where PAF was found to stimulate platelets to synthesize TXB_2, serotonin and platelet factor 4, it was suggested that TXB_2 release into the circulation during IgE-induced anaphylaxis in the rabbit may result, in part, from PAF stimulation of thromboxane synthesis by circulating rabbit platelets. However, this arachidonic acid metabolite was not necessary to alter circulating blood cells or platelet factor 4 secretion but may have served to reduce the IgE-induced anaphylactic reaction.

In cultured rat mesangial cells, PAF stimulated arachidonic acid release as well as PGE_2 synthesis. At the same time, PAF caused contraction of mesangial cells with a dose–response and time-course parallel to that for PGE_2 production. The PAF-elicited contraction of mesangial cells was enhanced when PGE_2 synthesis was inhibited. It was suggested that the production of glomerular prostaglandins may be an important facet of glomerulonephritis [79].

Arachidonic acid metabolites also play an important role in PAF-induced coronary vasoconstriction and reduced cardiac contractility in the isolated perfused rat heart [84]. Both cyclooxygenase- and lipoxygenase-derived arachidonic acid metabolites were released into the cardiac effluent perfusate in response to PAF challenge. These metabolites of arachidonic acid included $PGF_{2\alpha}$, PGE_2, 6-ketoPGF$_{1\alpha}$, TXB_2, LTB_4 and LTC_4. Through the use of inhibitors of lipoxygenase and cyclooxygenase and a leukotriene receptor antagonist, these authors demonstrated that LTC_4 released during PAF challenge was largely responsible for the coronary vasoconstriction induced following infusion of PAF. There was no information presented, however, as to whether a PAF receptor antagonist inhibited the cardiac effects of PAF. The possibility that PAF may act directly on target cells causing cardiac haemodynamic changes in the perfused heart was not ruled out [84].

In human [77] and guinea pig eosinophils [78], isolated from either the peritoneal cavity or bronchoalveolar lavages, PAF has been found to stimulate synthesis of LTC_4, LTB_4 and TXB_2. Human eosinophils produce primarily LTC_4, a powerful airway smooth-muscle constrictor [86], and 15-HETE, a substance thought to be responsible for excessive airway mucus production [87]. The finding that PAF induces synthesis of leukotrienes from lung cells may explain some of the pathophysiological roles of PAF in allergic respiratory diseases. Supporting this contention was an early observation [88] that PAF-induced rapid pulmonary vasoconstriction and oedema in isolated lungs was mediated through the action of LTD_4 and LTC_4, which were identified in the lung effluent perfusate after stimulation with PAF. Lipoxygenase-derived products of arachidonic acid also may mediate PAF-induced neutrophil aggregation and release of granule-associated enzymes [74–76].

The mechanisms by which PAF stimulates arachidonic acid release have been investigated extensively in various types of cells [56,63,80,89–93]. Using the relatively non-specific phospholipase A_2 inhibitors mepacrine and 2-(*p*-amylcinnamoyl)amino-4-chlorobenzoic acid [63,71] and using [^{14}C]arachidonic acid-labelled membranes as endogenous substrate and dioleoylphosphatidyl[^{14}C]ethanolamine as an exogenous substrate [80], it was demonstrated that phospholipase A_2 was responsible for the release of arachidonic acid in response to PAF. Through the use of EGTA and TMB-8 (an inhibitor of intracellular calcium mobilization), it was demonstrated that PAF-induced activation of phospholipase A_2 in neutrophils was dependent upon intra-

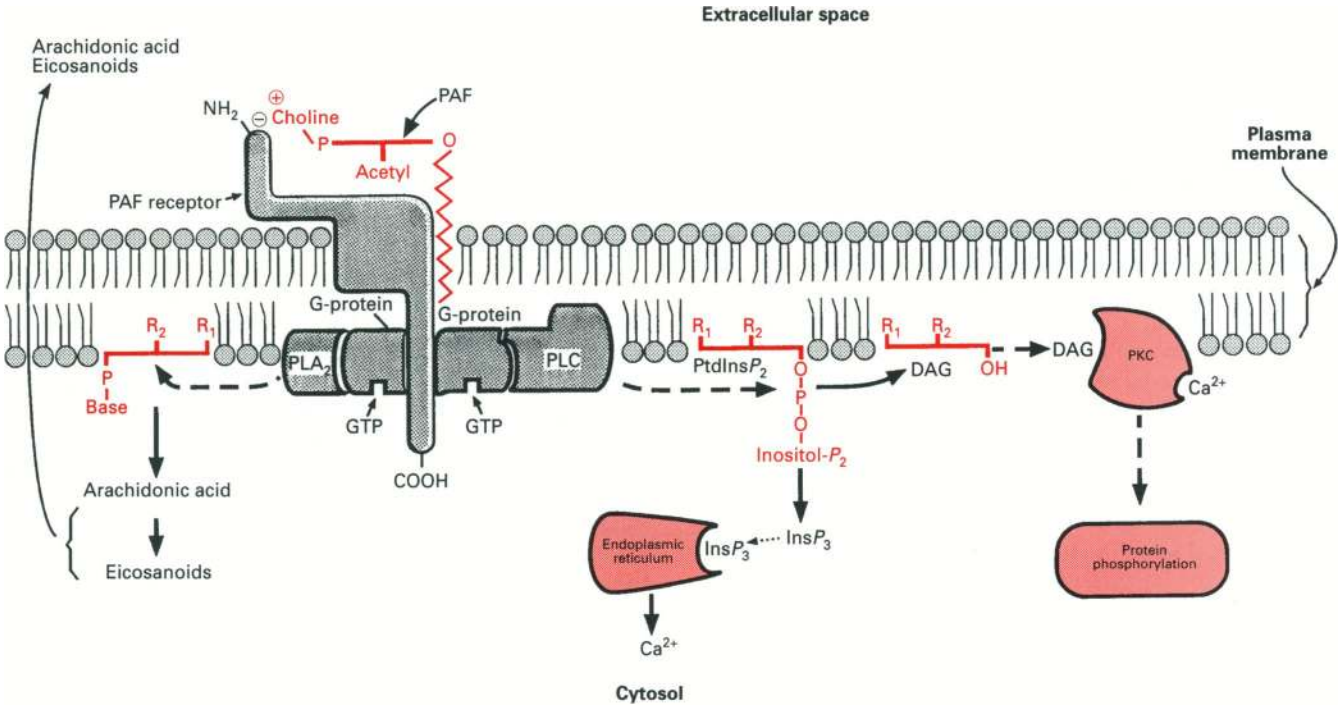

Figure 4 Schematic representation of PAF receptor-mediated intracellular signalling mechanisms

Recent studies have suggested that protein tyrosine phosphorylation (not shown) may also play a role in PAF signalling mechanisms [71,93,130–133].

cellular but not extracellular Ca^{2+} [63]. In cultured Kupffer cells, however, PAF-induced activation of phospholipase A_2 depended on extracellular Ca^{2+} [33,93]. In addition, PAF induces activation of phospholipase A_2 through a protein kinase C-dependent mechanism [59,90,91,93]. Pertussis toxin pretreatment abolished PAF-induced arachidonic acid release, suggesting a G-protein involvement in this event [60,63,90]. The stimulatory effect of PAF on phospholipase A_2 may also be regulated by intracellular cyclic AMP levels [56,92].

PAF not only stimulates arachidonic acid release from membrane phospholipids but also promotes incorporation of arachidonic acid into phospholipids. PAF stimulated the incorporation of [1-^{14}C]arachidonic acid in a Ca^{2+}-independent fashion most significantly into phosphatidylinositol and phosphatidylcholine in guinea pig [94] as well as in human neutrophils [95,96]. Moreover, PAF did not alter the distribution of [1-^{14}C]arachidonic acid in the various molecular species of phosphatidylcholine (diacyl, alkylacyl and alkenylacyl species) after brief incubation intervals, suggesting that the increased formation of [1-^{14}C]arachidonylphosphatidylcholine was not derived from the added PAF, e.g. alkylacyl-phosphatidylcholine formation with an ether linkage at the *sn*-1 position. The mechanism of the stimulatory effect of PAF on acylation of phospholipids has not been elucidated completely. Both increased fatty acid uptake and increased availability of lysophospholipids following phospholipase A_2 activation may contribute to the increased phospholipid acylation induced by PAF [96]. However, other mechanisms such as the increased activity of fatty acyl-CoA synthetase and acyltransferase, two enzymes essential for the acylation of phospholipids during PAF stimulation, deserve to be considered.

Arachidonic acid can be generated directly or indirectly by agonist activation of phospholipases and may act as a signalling element in various cellular reactions. In addition to serving as a precursor for eicosanoid synthesis, a function which has considerable pathophysiological significance, arachidonic acid has been implicated in a variety of biochemical responses such as activation of protein kinase C and adenylate cyclase, and the regulation of intracellular calcium concentrations [97]. Also, arachidonic acid was found to play an important role in the regulation of K^+ channel opening in cardiac and smooth muscle [98]. Moreover, it has been suggested that there exists a regulatory relationship between arachidonic acid and phosphoinositide hydrolysis. It has been demonstrated that arachidonic acid causes a dose-dependent increase in the accumulation of inositol phosphates, including InsP_3, InsP_2, and Ins$P$, in cultured astrocytes [99]. Inositol phosphate formation following application of carbachol or noradrenaline was additive with arachidonic acid, whereas a similar response evoked by PAF or ATP was not additive with arachidonic acid.

Phosphoinositide turnover

It has become clear that one of the key events in PAF-mediated signalling mechanisms is the hydrolysis of phosphatidylinositol 4,5-bisphosphate, by a specific phospholipase C, yielding two second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate. DAG was found to activate protein kinase C leading to phosphorylation of various substrates [100,101] whereas InsP_3 mobilizes intracellular calcium [102,103] (Figure 4). PAF has been found to stimulate the hydrolysis of phosphatidylinositol in a wide variety of cell types including rabbit [104,105], human [106], and horse [107,108] platelets, smooth muscle cells [109], hepatocytes [110,111], macrophages [30,112,113], neutrophils [114], endothelial cells [115], human keratinocytes [116], glomerular mesangial cells [60,117] and

Kupffer cells [118,119]. It was first reported that PAF stimulated metabolism of inositol phospholipids and phosphatidic acid in washed rabbit platelets [104]; PAF caused a 15–20% decrease in the PtdIns level within 15 s with a dramatic four-fold increase in phosphatidic acid. The effect of PAF on the metabolism of phosphoinositide was quite specific since other major classes of phospholipids were not affected. If [^{32}P]P_i was present in the medium, PAF enhanced significantly the incorporation of radioactivity into the PtdIns*P*, PtdInsP_2 and phosphatidic acid fractions within 1 min while the incorporation of [^{32}P]P_i into phosphatidylinositol increased thereafter. In ^{32}P-labelled hepatocytes [110], PAF, at a concentration of 5×10^{-10} M, caused a 30–40 % decrease in [^{32}P]PtdInsP_2 within 10 s. The ^{32}P content of the PtdIns*P* and PtdIns fractions also decreased but at a slower rate. With horse platelets prelabelled with [^{32}P]P_i, it was shown that PAF initiated a rapid formation of labelled phosphatidic acid followed by an increase in phosphatidylinositol.

PAF has potent stimulatory effects on macrophages [30,112]. Macrophages stimulated with PAF produced several inositol phosphates, including Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, and as a consequence, the intracellular level of calcium was elevated up to 290±27% of the basal level (82.7±12 nM) [30]. It should be pointed out that the metabolites [e.g. Ins(1,4,5)P_3] of phosphoinositide metabolism may not always be responsible for the PAF-induced increase in cytosolic calcium. In Kupffer cells, for example, more than 90% of cytosolic free Ca^{2+} is due to extracellular Ca^{2+} influx rather than mobilization of Ca^{2+} from intracellular sites in response to PAF stimulation [93] although PAF also stimulates metabolism of phosphoinositide within the same time frame [118,119]. On the other hand, as a consequence of metabolism of phosphoinositide, PAF increases DAG levels [30], stimulates protein kinase C activity [93], and causes protein phosphorylation [30]. In an attempt to investigate the relationship between inositol lipid metabolism and the production of reactive oxygen intermediates (ROI), Huang et al. [112] found that PAF treatment led to a rapid increase in [^{3}H]InsP_3 levels and 1,2-diacyl[^{3}H]glycerol levels in bone marrow-derived macrophages labelled with [^{3}H]inositol or [^{3}H]glycerol, respectively. This response to PAF was followed by an increase in the production of ROI. Pretreatment of macrophages with phorbol ester or pertussis toxin attenuated both PAF-induced [^{3}H]inositol phosphate production and ROI production. Phorbol ester, a protein kinase C activator, but not calcium ionophore A23187, stimulated ROI production. It was proposed, therefore, that the DAG formed and consequent protein kinase C activation following PAF stimulation was responsible for the increased production of ROI. Protein kinase C activation also may mediate other PAF-induced cellular responses such as arachidonic acid release and eicosanoid production and protein tyrosine phosphorylation [93].

Using a double labelling technique, Okayasu et al. [111] demonstrated in primary cultured rat hepatocytes that PAF stimulated the breakdown of phosphoinositides via phospholipase A_2. Addition of PAF to cells labelled with [^{14}C]glycerol and [^{3}H]arachidonic acid caused a transient decrease in [^{14}C]glycerol-labelled PtdIns and an increase in [^{14}C]glycerol-labelled lysoPtdIns. [^{3}H]Arachidonic acid-labelled PtdIns decreased in a time-dependent fashion and the radioactivity in phosphatidylcholine, phosphatidylethanolamine and other major phospholipids was not affected by the addition of PAF. The [^{3}H]arachidonate/[^{14}C]glycerol ratio decreased significantly in PtdIns and [^{3}H]arachidonic acid appeared within 10 s upon stimulation with PAF. Also, PAF increased [^{3}H]inositol-labelled lysoPtdIns in *myo*-[^{3}H]inositol-labelled hepatocytes in the absence of an accumulation of [^{3}H]inositol-labelled inositol phosphates. In addition, a precursor–product relationship was detected between PtdIns and lysoPtdIns in [^{32}P]P_i-labelled hepatocytes stimulated with PAF. These observations suggested that PAF stimulated the metabolism of phosphoinositides via activation of phospholipase A_2 rather than via the PtdIns cycle or a polyphosphoinositide turnover mechanism in primary cultured hepatocytes.

Calcium flux

PAF causes an elevation of cytosolic free calcium in various cells such as platelets [120,121], neutrophils [90,114,122,123], macrophages [30,69,124], mesangial cells [111,117], vascular smooth muscle cells [109,125], endothelial cells [126], Kupffer cells [93,118] and neuronal cells [38]. There are at least two mechanisms involved in PAF-induced increases in cytosolic free calcium: (a) calcium influx occurs through a membrane-associated calcium channel regulated by PAF receptors or by signalling molecules generated intracellularly (e.g. metabolites of arachidonic acid) and (b) mobilization of calcium is instigated from intracellular stores in response to the intracellular second messenger InsP_3 produced during PAF receptor stimulation.

Lee et al. [120,121] first reported that PAF induced a calcium influx in rabbit platelets in a dose-dependent manner. The PAF-stimulated $^{45}Ca^{2+}$ influx in rabbit platelets could be blocked by verapamil, a calcium channel blocker, and was dependent upon extracellular [Ca^{2+}]. Calcium mobilization was independent of cyclo-oxygenase products of arachidonic acid metabolism but was inhibited significantly by mepacrine, *p*-bromophenacyl bromide, eicosatetraynoic acid and nordihydroguaiaretic acid. These observations suggest that lipoxygenase-derived metabolites of arachidonic acid produced in response to PAF stimulation may mediate the PAF-induced calcium uptake in rabbit platelets [121]. In mesangial cells [117], PAF increased cytosolic free calcium within 10 s, due to both a release of Ca^{2+} from intracellular storage sites as well as an influx of extracellular Ca^{2+}. The rise in cytosolic free [Ca^{2+}] in response to PAF stimulation was attributed to an increased production of InsP_3.

In vascular smooth muscle cells, PAF stimulated the hydrolysis of phosphoinositide and a rapid efflux of $^{45}Ca^{2+}$ from preloaded cells [109]. In an attempt to elucidate the mechanism for desensitization of the PAF response, it was demonstrated that PAF elicited a transient, dose-dependent increase in cytosolic free Ca^{2+} in vascular smooth muscle cells preloaded with fura-2 and an increase in InsP_3 and InsP_4 levels [125]. Pretreatment of the cells with PAF or PMA attenuated subsequent PAF- and angiotensin II-induced Ca^{2+} mobilization but not vasopressin-stimulated Ca^{2+} mobilization. The authors proposed that both homologous and heterologous desensitization is mediated by PAF-stimulated phosphoinositide hydrolysis and DAG formation [125]. The PAF-induced increase in cytosolic free Ca^{2+} seemed to be independent of InsP_3 since the peak of Ca^{2+} concentration was reached before there was a significant increase in the amount of InsP_3. The effect of PAF was dependent upon extracellular calcium. In vascular endothelial cells preloaded with fura-2, however, it was demonstrated that PAF elicited an elevation of cytosolic free Ca^{2+} released primarily from intracellular stores [126].

A PAF-elicited increase in cytosolic free Ca^{2+} in rabbit neutrophils preloaded with fura-2 was inhibited by pretreatment of the cells with fMet-Leu-Phe [123]. This finding was explained as an increased production of endogenous PAF which bound to and inactivated the PAF receptor. Pretreatment of the cells with PMA, a potent protein kinase C activator, abolished completely the rise in intracellular free Ca^{2+} induced by PAF. This effect of

PMA appeared to be mediated by protein kinase C activation, since the protein kinase C inhibitor H-7 attenuated the inhibitory effect of PMA [123]. In an attempt to assess the relative contribution of Ca^{2+} released from intracellular stores and Ca^{2+} influx from the extracellular medium to the elevation of intracellular Ca^{2+} during neutrophil activation, it was found that Ca^{2+} release from intracellular stores was rate-limiting for the PAF- and fMet-Leu-Phe-induced increase in intracellular free Ca^{2+} [122].

With murine peritoneal macrophages preloaded with fura-2/AM, Prpic et al. [30] demonstrated that PAF elevated intracellular levels of Ca^{2+} to $290 \pm 27\%$ of basal levels (82.7 ± 12 nM). Using colour-enhanced computer images of the 340nm:380 nm fluoresence ratio of a single macrophage, Prpic showed that increases in intracellular Ca^{2+} were observed first in a submembranous area of the macrophage. In single mouse macrophages preloaded with fura-2, Randriamampita and Trautmann [124] observed that a pulse administration of PAF caused a biphasic increase in intracellular free [Ca^{2+}], including an initial transient and then a more sustained increase in intracellular Ca^{2+}. The initial transient phase, which lasted for a few seconds, was independent of extracellular Ca^{2+} concentration, suggesting a release of Ca^{2+} from intracellular stores. The second phase of this response, which lasted for several minutes, was sensitive to extracellular Ca^{2+} concentrations and was probably due to an influx of Ca^{2+} through the plasma membrane. Also, similar biphasic responses to PAF were observed in cultured rat mesangial cells [127]. Human monocytic leukaemic U-937 cells [128] and HL-60 cells [129], when differentiated with dimethyl sulphoxide to a macrophage-like state, express specific PAF receptors and respond to PAF stimulation by increasing their intracellular free Ca^{2+} levels [128]. This response to PAF depends upon extracellular Ca^{2+} concentrations and can be blocked by the receptor antagonist CV-3988 but not by calcium channel blockers such as nifedipine or verapamil. Both PAF-induced calcium mobilization and phosphoinositide metabolism were insensitive to pertussis toxin, but sensitive to the phospholipase C inhibitor, manoalide.

Protein tyrosine phosphorylation

Recent studies have demonstrated that PAF stimulates tyrosine phosphorylation of numerous cellular proteins in platelets [130,131], neutrophils [132], and liver macrophages [93,133]. PAF-induced tyrosine phosphorylation is extracellular Ca^{2+}-dependent and may be mediated by G-protein(s) and PKC activation [93,132]. Although it has been suggested that a tyrosine kinase is involved in the PAF-stimulated phosphoinositide turnover in platelets [130] and arachidonic acid metabolism in macrophages [71], the molecular mechanism for the interaction between the tyrosine kinase and the phospholipases is not understood. Characterization of the tyrosine-phosphorylated proteins in platelets led to the identification of a 60 kDa phosphoprotein as the proto-oncogene product $pp60^{c\text{-}src}$ [131]. It will be of interest to determine the functional consequence of the tyrosine phosphorylation of $pp60^{c\text{-}src}$ protein in the PAF-stimulated platelets. Also, PAF may play a role in gene expression. It was found that PAF is capable of inducing transcription of the nuclear proto-oncogenes c-*fos* and c-*jun* in B cells [134,135]. Since the cloned PAF receptor contains several tyrosine residues in its intracellular loops and tail (Figure 3) [14,16], it will be of interest to examine whether homologous down-regulation of PAF receptors [55] and PAF-induced protein tyrosine phosphorylation are related. Vanadate, an inhibitor of protein tyrosine phosphatase, stimulates tyrosine phosphorylation of numerous cellular proteins and induces a decrease in the number of the surface PAF receptors [133]. Both effects of vanadate can be inhibited by genistein, a putative tyrosine kinase inhibitor, suggesting that protein tyrosine phosphorylation plays a role, directly or indirectly, in the regulation of the surface expression of PAF receptors.

Hepatic actions of PAF

PAF, when infused into the perfused rat liver, resulted in a several-fold increase in glucose output in the effluent perfusate [110,136]. In contrast, when a 500-fold higher concentration of 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine or the stereoisomer 3-*O*-alkyl-2-acetyl-*sn*-glycero-1-phosphocholine was infused, no increased glycogenolysis was observed. The glycogenolytic effect of PAF depends upon both ligand and the Ca^{2+} concentration in the perfusate. Surprisingly, PAF failed to stimulate glycogenolysis in isolated hepatocytes although adrenaline and glucagon elicited glucose output in the same preparation [137]. In an attempt to elucidate the mechanism by which PAF causes glycogenolysis in the perfused liver, it was observed that infusion of PAF caused a transient increase in portal vein presure concomitant with the increase in glycogenolysis [138]. The vascular and metabolic responses were correlated closely, displaying similar dose dependence and similar attenuation in response to a reduction in perfusate Ca^{2+} concentration. The activity of glycogen phosphorylase *a* and the tissue ADP level were increased significantly in the perfused liver in response to PAF. Furthermore, nitric oxide, a compound which relaxes vascular smooth muscle, was found to inhibit or to abolish PAF-induced increases in portal vein pressure, oxygen consumption and, most importantly, hepatic glucose output in the perfused rat liver [139]. In contrast to its effect on PAF-induced hepatic responses, nitric oxide inhibited only the haemodynamic but not the glycogenolytic effects of phenylephrine, which acts directly on hepatocytes to induce glycogenolysis and glucose production [139]. Based on these observations, it was proposed that the glycogenolytic effect of PAF in the perfused liver was a result of the haemodynamic effects of PAF, rather than a direct effect of the agonist on the hepatocyte (Figure 5). Further evidence suggested that hepatic reticuloendothelial cells, e.g. endothelial and/or Kupffer cells, may play an important role in the PAF-induced hepatic responses observed in the perfused liver: heat-aggregated IgG [140], which stimulates reticuloendothelial cells in the liver, causes hepatic responses similar to those of PAF in the perfused liver; [^{3}H]PAF infused into the perfused liver was localized specifically in small portal venules instead of in parenchymal cells [141]; appropriately stimulated isolated Kupffer cells synthesize PAF and actively metabolize this potent phospholipid [37,142]; and, finally, isolated rat hepatocytes, the glycogen-storing cells in the liver, lack detectable specific PAF-binding sites (W. Chao and M. S. Olson, unpublished work).

In an effort to elucidate the important role of Kupffer cells in the hepatic actions of PAF, the specific receptor for PAF and the regulatory characteristics of the receptor have been investigated [33,55,56,59]. It was found that isolated Kupffer cells possess a large number of high-affinity receptors for PAF [33]. The PAF receptor identified in Kupffer cells is functionally active since it mediates arachidonic acid release and eicosanoid production [33,56,59,143]. It has been proposed that non-parenchymal cells, in response to certain stimuli, release biologically active eicosanoids which then act on parenchymal cells to stimulate glycogenolysis [144]. The release of the biologically active eicosanoids from non-parenchymal cells was suggested to

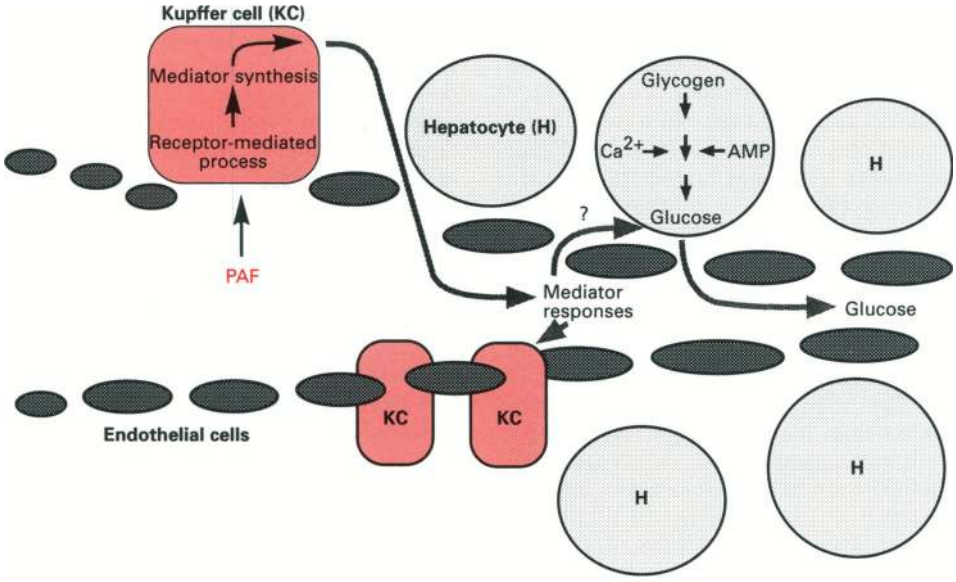

Figure 5 Schematic representation of the intercellular signalling mechanism for PAF in the liver

When infused into the rat liver through the portal vein, PAF induces glycogenolysis and vasoconstriction as illustrated by the narrowing sinusoid. The glycogenolytic effect of PAF is indirect, requiring interaction of PAF with Kupffer cells and/or endothelial cells resulting in severe hepatic vasoconstriction. It has been proposed that this mediator-induced haemodynamic response produces a transient ischaemia which initiates the glycogenolytic response, probably through an elevation of tissue AMP levels and possibly of intracellular free calcium levels in hepatocytes.

mediate both PAF- [143] and endotoxin- [145] stimulated glycogenolysis in the perfused liver since the cyclo-oxygenase inhibitors aspirin and indomethacin blocked the glycogenolytic action of these factors [143,145,184] (Figure 5). In addition, media obtained from aspirin-treated Kupffer cells or endothelial cells had no effect on glucose production by parenchymal cells [144]. However, this proposal has been questioned by the observation that ibuprofen (50 mM), a specific cyclo-oxygenase inhibitor, does not inhibit PAF-induced glycogenolysis and vasoconstriction significantly although it abolishes eicosanoid production in the perfused liver [85]. Nevertheless, it is probable that the hepatic glycogenolytic action of PAF observed in the perfused liver is indirect, requiring interaction between the parenchymal cells, i.e. hepatocytes, and the non-parenchymal cells, i.e. Kupffer cells and endothelial cells (Figure 5).

The haemodynamic and hyperglycaemic effects of PAF can be regulated by β-adrenergic receptor stimulation [146,147]. Infusion of isoproterenol, a β-adrenergic receptor agonist, into the perfused rat liver attenuated the glycogenolytic stimulation caused by PAF. The regulatory effect of the β-adrenergic agonist was believed not to be mediated by a cyclic AMP increase in 'parenchymal' cells since glucagon, which increases hepatic cyclic AMP levels to a far greater extent than does isoproterenol, had no effect on the glycogenolytic response of the liver to PAF [146]. It was suggested that the mechanism by which isoproterenol regulates the glycogenolytic effect of PAF in the perfused liver may involve interaction of the β-agonist with non-parenchymal cells [56,146,147]. Further studies demonstrated that isoproterenol attenuates the subsequent PAF-stimulated biological effects, including arachidonic acid release and cyclo-oxygenase-derived eicosanoid production in isolated Kupffer cells. The regulatory effect of isoproterenol is highly specific and involves a β_2-adrenergic receptor- and a cyclic AMP-mediated mechanism [56]. Long-term incubation of Kupffer cells with cyclic AMP analogues or forskolin down-regulates the surface expression of PAF receptors [56].

Ligand binding studies indicated that PAF down-regulates the surface expression of its own receptor in cultured Kupffer cells [55]. Both the rate of loss and the maximal extent of loss of the receptors were dependent upon PAF concentration. With receptor synthesis inhibited by cycloheximide in the absence of PAF, the half-life of the surface PAF receptor was about 4 h, suggesting that the turnover of the PAF receptors on the plasma membrane is continuous. Through the use of cycloheximide [55] or actinomycin D (our unpublished work), it was demonstrated that PAF receptors are not recycled and that the restoration of lost or inactivated PAF receptors requires newly synthesized protein. The fact that both cycloheximide and actinomycin D prevent the restoration of PAF receptors suggests that tissue responsiveness to PAF may be regulated by both transcription and translation. Also, surface expression of PAF receptors in Kupffer cells is down-regulated by protein kinase C activation [59,93]. The effect of protein kinase C is specific and transient and, as a consequence, PAF-mediated arachidonic acid release and eicosanoid production are attenuated. Also, protein kinase C may be involved in the stimulatory signal transduction between the PAF receptor and the phospholipase(s) responsible for the release of arachidonic acid and subsequent production of eicosanoids. This contention is based on the observations that both down-regulation of protein kinase C and a protein kinase C inhibitor attenuate PAF-stimulated arachidonic acid release as well as eicosanoid production [59,93].

SUMMARY

During the past two decades, studies describing the chemistry and biology of PAF have been extensive. This potent phosphoacylglycerol exhibits a wide variety of physiological and pathophysiological effects in various cells and tissues. PAF acts, through specific receptors and a variety of signal transduction systems, to elicit diverse biochemical responses. Several important future directions can be enumerated for the

characterization of PAF receptors and their attendant signalling mechanisms. The recent cloning and sequence analysis of the gene for the PAF receptor will allow a number of important experimental approaches for characterizing the structure and analysing the function of the various domains of the receptor. Using molecular genetic and immunological technologies, questions relating to whether there is receptor heterogeneity, the precise mechanism(s) for the regulation of the PAF receptor, and the molecular details of the signalling mechanisms in which the PAF receptor is involved can be explored. Another area of major significance is the examination of the relationship between the signalling response(s) evoked by PAF binding to its receptor and signalling mechanisms activated by a myriad of other mediators, cytokines and growth factors. A very exciting recent development in which PAF receptors undoubtedly play a role is in the regulation of the function of various cellular adhesion molecules [148,185,186]. Finally, there remain many incompletely characterized physiological and pathophysiological situations in which PAF and its receptor play a crucial signalling role. Our laboratory has been active in the elucidation of several tissue responses in which PAF exhibits major autocoid signalling responses, e.g. hepatic injury and inflammation [149], acute and chronic pancreatitis [150,151], and cerebral stimulation and/or trauma [39]. As new experimental strategies are developed for characterizing the fine structure of the molecular mechanisms involved in tissue injury and inflammation, the essential role of PAF as a primary signalling molecule will be affirmed. Doubtless the next 20 years of experimental activity will be even more interesting and productive than the past two decades.

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