Role of Platelet-Activating Factor in Cardiovascular Pathophysiology

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Montrucchio, Guiseppe, Guiseppe Alloatti, and Giovanni Camussi. Role of Platelet-Activating Factor in Cardiovascular Pathophysiology. *Physiol Rev* 80: 1669–1699, 2000.—Platelet-activating factor (PAF) is a phospholipid mediator that belongs to a family of biologically active, structurally related alkyl phosphoglycerides. PAF acts via a specific receptor that is coupled with a G protein, which activates a phosphatidylinositol-specific phospholipase C. In this review we focus on the aspects that are more relevant for the cell biology of the cardiovascular system. The in vitro studies provided evidence for a role of PAF both as intercellular and intracellular messenger involved in cell-to-cell communication. In the cardiovascular system, PAF may have a role in embryogenesis because it stimulates endothelial cell migration and angiogenesis and may affect cardiac function because it exhibits mechanical and electrophysiological actions on cardiomyocytes. Moreover, PAF may contribute to modulation of blood pressure mainly by affecting the renal vascular circulation. In pathological conditions, PAF has been involved in the hypotension and cardiac dysfunctions occurring in various cardiovascular stress situations such as cardiac anaphylaxis and hemorrhagic, traumatic, and septic shock syndromes. In addition, experimental studies indicate that PAF has a critical role in the development of myocardial ischemia-reperfusion injury. Indeed, PAF cooperates in the recruitment of leukocytes in inflamed tissue by promoting adhesion to the endothelium and extravascular transmigration of leukocytes. The finding that human heart can produce PAF, expresses PAF receptor, and is sensitive to the negative inotropic action of PAF suggests that this mediator may have a role also in human cardiovascular pathophysiology.
I. INTRODUCTION

Platelet-activating factor (PAF) is one of the most potent and versatile mediators found in mammals. It was originally described as “a soluble factor” involved in leukocyte-dependent histamine and serotonin release from platelets (177, 379). In 1972, Benveniste et al. (31) demonstrated that this soluble factor was released from rabbit basophils after IgE stimulation and coined the term PAF. Several reports followed describing the lipid nature of PAF (29, 32, 113, 332). Almost concomitantly, a factor with properties similar to PAF was isolated from the renal medulla by Muirhead and co-workers (44, 303, 305). This factor was named antihypertensive polar renal lipid because of its ability to lower blood pressure in the Goldblatt rat model of hypertension. In 1979, three independent groups (33, 44, 113) demonstrated that a semisynthetic phosphoacylglycerol, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, had physicochemical as well as biological properties indistinguishable from those of naturally occurring PAF/anti hypertensive polar renal lipid. Hanahan et al. (164) characterized by gas-liquid chromatography and mass spectral analysis the chemical structure of PAF released by IgE-sensitized rabbit basophils as a 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine. Although PAF continues to be the common term used, it is a misnomer, because it identifies only the platelet effect of this mediator. PAF is now considered a phospholipid with diverse and potent physiological effects that belongs to a family of biologically active, structurally related alkyl phosphoglycerides (48, 79, 100, 163, 280, 337, 384, 385, 442, 446). PAF is thought to be a mediator of cell-to-cell communication, which may function either as an intercellular or an intracellular messenger (100). Some of its actions are achieved at concentrations as low as 10^{-12} M and include events relevant for the development of several pathological and physiological processes. Numerous cell types and tissues have been shown to produce PAF upon appropriate stimulation (47). In particular, PAF is produced by a variety of cells that may participate in the development of inflammatory reaction such as monocytes/macrophages, polymorphonuclear neutrophils (PMN), eosinophils, basophils, and platelets (50, 79, 260, 428). In addition, human endothelial cells were found to produce PAF after stimulation by several inflammatory mediators (458) including thrombin (69, 179, 336, 474), angiotensin II (69), vasopressin (69), leukotrienes C4 and D4 (276), histamine (276), bradykinin (276), elastase (80), cathepsin G (80), hydrogen peroxide (145, 255), plasmin (289, 298), interleukin (IL)-8 (21) and IL-1α, or tumor necrosis factor (TNF)-α (61, 64, 60, 73, 228, 229, 286). Cardiomyocytes have been also reported to synthesize PAF under appropriate stimulation (200). Most of the cells that produce PAF also possess PAF receptors (185, 232, 403, 470) and are target for PAF action. In vitro, PAF promotes the aggregation, chemotaxis, granule secretion, and oxygen radical generation from leukocytes and the adherence of leukocytes to the endothelium (51, 81, 322, 373, 480). PAF increases the permeability of endothelial cell monolayer (62), stimulates the contraction of smooth muscle (139, 209, 396) and myometrium (292, 414), and has negative inotropic, arrhythmogenic effects on cardiac muscle (11, 30, 71, 251, 352, 353).

In this paper we review the molecular and cellular basis for the structural and functional diversity of PAF molecules, the different biosynthetic and catabolic pathways of PAF, and the signal transduction triggered by the engagement of PAF receptor. Moreover, we describe the systemic and local effects of PAF administration in vivo and the mechanical and electrophysiological effects of PAF on isolated perfused heart, atrium, and papillary muscle and on cultured cardiomycocytes. We also examine the microcirculatory effect of PAF and its role in the interaction between endothelial and inflammatory cells. Finally, the involvement of PAF in cardiovascular pathophysiological processes such as shock syndromes, ischemia-reperfusion injury, atherogenesis, and neoangiogenesis is discussed in the light of the biological properties of this mediator and of the effect of PAF-receptor antagonists.

II. MOLECULAR HETEROGENEITY OF PLATELET-ACTIVATING FACTOR AND FUNCTIONAL IMPLICATIONS

Although PAF is generally considered as a single molecular entity with a wide spectrum of diverse and potent biological properties, it is now clear that there are a variety of structurally related phospholipid molecules of biological origin that share many of the same physiological activities (for review, see Ref. 280). However, the chemical structure strictly influences the biological potency of PAF (384). The full expression of its biological potency requires an ether linkage at the sn-1 position of the glycero backbone, a short acyl chain, usually an acetyl residue, at the sn-2 position, and the polar head group of choline or ethanolamine at the sn-3 position (280, 384). The length of the acyl chain at the sn-1 position and the number of double bonds have modest effects on its biological potency. In contrast, the presence of an ether linkage at the sn-1 position of glycerol or a three-carbons longer acyl chain at sn-2 position significantly diminishes and changes the biological properties. Mass spectral analysis of PAF synthesized by human PMN showed the presence of multiple molecular species of alkyl PAF including 15:0-, 16:0-, 17:0-branched chain, 17:0-, 18:0-, 18:1-, 18:2-, and
22:2-PAF (262, 333, 452, 453). Human PMN also produce acyl-PAF that represents 13–25% of total PAF synthesized by these cells (301, 426). More recently, it has been suggested that the acyl-PAF may be the major molecular species of PAF produced by several cell types including human pulmonary mastocytes (425, 427) and vascular endothelium (194, 428, 457). Perfused rat and guinea pig hearts (312) produce vinyl-PAF. Other species of biologically active acetylated glycerolipids are the plasmalogen analogs of PAF such as 1-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphocholine and 1-alk-1′-enyl-2-acetyl-sn-glycero-3-phosphoethanolamine and the 1-alkyl-2-acetyl-sn-glycerols, the neutral lipid precursor of PAF. Such plasmalogens even if less active than PAF itself can act synergistically with PAF (262, 385). The biological activities of 1-alkyl-2-acetyl-sn-glycerols and their neutral lipids to PAF. However, 1-alkyl-2-acetyl-sn-glycerols and their metabolic products have been shown to possess intrinsic biological activities (437) including differentiation of HL-60 cells (281), attenuation of dia-cyglycerol-induced activation of protein kinase C (PKC), and activation of macrophages (466). Finally, the 1-alkyl-2-acetyl-sn-glycero-phosphate, an analog of phosphatidic acid, can act as a calcium ionophore (63). The different molecular species of PAF have different potency on platelet activation (280). However, platelet bioassay does not necessarily reflect the biological potential of a given molecular species on other cell types or tissues. For instance, it has been shown that the presence of a single double bond in the alkyl chain at the sn-1 position significantly alters the cardiac activity by increasing 50-fold the negative inotropism and 100-fold coronary vasoconstriction but has little effect on the stimulation of platelets (280). McManus et al. (280) have recently reviewed the differences in the rank orders of potency of alkyl and acyl-PAF molecules in vitro on rabbit platelets, human PMN, and isolated guinea pig heart, and in vivo, when injected into rabbit on thombocytes, leukemia, and right ventricular hypertension. It has been suggested that the differences in the biological properties of various molecular species of PAF may depend on differences in the biophysical properties as critical micellar concentration or albumin binding affinity or on the existence of more than one receptor subtype for PAF.

III. METABOLISM OF PLATELET-ACTIVATING FACTOR

A. PAF Biosynthetic Pathways

The molecular heterogeneity of PAF possibly depends on the multiple enzymatic steps involved in its synthesis and degradation by different cell types.

PAF is synthesized by two main pathways in a variety of tissues and cells. The “remodeling pathway” is mainly involved in the synthesis of PAF by stimulated inflammatory cells (14, 240, 319). This pathway requires a tightly coupled reaction of phospholipase (PL) A2 and acetyl CoA:1-alkyl-sn-glycero-3-phosphorylcholine 2-O-acetyltransferase (464). The activation of PLA2 (461) determines the hydrolysis of membrane phospholipids to generate a variety of 2-lysophospholipids (e.g., 1-alkyl-2-lyso-glycero-3-phosphocholine, lyso-PAF). These 2-lysophospholipids are the substrate of acetyl CoA:1-alkyl-sn-glycero-3-phosphorylcholine 2-O-acetyltransferase, which catalyzes the transfer of the acetyl moiety from acetyl CoA to the free hydroxyl at sn-2 position. In addition to a direct decylation of membrane glycerophospholipids, another pathway for the generation of 2-lysophospholipids has been recently described. Indeed, lyso-PAF can be obtained via a CoA-independent transacylation reaction between alkylacyl-glycerol-3-phosphocholine and the lysophospholipid acceptor formed via the action of a putative PLA2 (25, 106, 317, 386, 434, 445). This CoA-independent transacylase route accounts for the simultaneous PAF synthesis and mobilization of arachidonic acid, since it is specific for the arachidonate linked species of alkyl choline phosphoglycerides (354, 460). Figure 1 shows the enzymatic steps of the remodeling pathway.

The second biosynthetic pathway of PAF that is mainly operative in the kidney and in the central nervous system (65, 350, 384) has been termed “de novo pathway” (Fig. 2). This involves the synthesis of 1-O-alkyl-2-acetyl-glycerol, which is then converted to PAF by a specific dithiothreitol-insensitive CDP-choline:1-alkyl-2-acetyl-sn-glycero cholinephosphotransferase. Except for the insensitivity of cholinephosphotransferase to dithiothreitol, this pathway is analogous to that involved in the biosynthesis of lecithins (350). The direct precursors of PAF in this pathway are 1-alkyl-2-acetyl-sn-glycerols, formed via an acetylation-dephosphorylation sequence, which is catalyzed by acetyl CoA:1-alkyl-2-lyso-sn-glycero-3-phosphate acetyltransferase and by 1-alkyl-2-acetyl-sn-glycero-3-phosphate phosphohydrolase (239, 243, 244).

The enzymes of the remodeling pathway and de novo pathway have relative broad substrate specificities that provide a basis for heterogeneity in the molecular species of PAF produced by a given cell or tissue in response to a specific stimulus (280, 384).

B. PAF Catabolism

The synthesis and catabolism of this potent phospholipid autacoid are highly regulated. The final molecular composition of PAF in tissues and the expression of its biological activities depend also on the activation of cat-
abolic pathways. The most important enzyme in the limitation the PAF bioactivity is a PAF-specific acetylhydrolase (PAF-AH), which cleaves the short acyl chain at sn-2 position and forms the biologically inactive lyso-PAF. This enzyme is present in plasma (130) and in various tissues (43, 186, 242, 318, 392). The molecular cloning and characterization of the human plasma PAF-AH have been recently reported (415, 418). The PAF-AH activity found in human plasma circulates as a complex with low- (LDL) and high-density lipoproteins (HDL) (203, 391, 429). In addition to the extracellular enzyme, the molecular characterization of two intracellular enzyme, the molecular characterization of two intracellular PAF-AH has been reported (171–173). PAF-AH degrades also PAF-like oxidized phospholipids that were shown to bind PAF receptors (382) and have been implicated in the pathogenesis of atherosclerosis (174). The lyso-PAF is then reacylated by an acyl-CoA:1-radyl-sn-glycero-3-phosphorylcholine acyltransferase. The alkyl moiety of lyso-PAF is known to be cleaved to an aldehyde by a tetrahydropiridine-dependent alkyl monooxygenase (239). Alternatively, phospholipase D can hydrolyze phosphocholine moiety to produce an analog of phosphatidic acid or catalyze a phosphatase transfer by a transphosphatidylation reaction (7). The acyl PAF molecule can be also degraded by a PLA1, which hydrolyzes the long-chain fatty acyl residue esterified at the sn-1 position to produce 1-lyso-2-acetyl-glycero-3-phosphocholine (424). Recently, it has been shown that guinea pig hearts release acetyl hydrolase in the systemic circulation and that isolated ventricular myocytes are capable to take up PAF and catabolize it to inactive products (341, 423). The coronary artery bypass surgery has been shown to induce changes in serum PAF-acetyl hydrolase activity (314).

FIG. 1. Remodeling pathways for synthesis of platelet-activating factor (PAF). PAF synthesis is initiated by the activation of phospholipase A2 (PLA2), which may act on 1-O-alkyl-2-arachidonoyl glycerophosphocholine to yield lyso-PAF and free arachidonic acid. The lyso-PAF is acetylated by a specific lyso-PAF acetyltransferase using acetyl coenzyme A as a donor. Alternatively PLA2 may act on arachidonate-containing plasmalogen phosphatidylethanolamine to release free arachidonate. The lyso-phosphatidylethanolamine may act as an acceptor for arachidonate in a transacylation reaction from the PAF precursor with formation of lyso-PAF. Therefore, the activation of PLA2 is essential for generation of lyso-PAF and triggering the remodeling pathways. The activation of lyso-PAF acetyltransferase, which is modulated by a phosphorylation/dephosphorylation cycle, is a limiting factor for generation of biologically active PAF.

De Novo

FIG. 2. De novo pathway for synthesis of PAF. In this pathway, 1-O-alkyl-sn-glycero-3-phosphate is acetylated by 1-O-alkyl-sn-glycero-3-phosphate:acetyl CoA acetyltransferase and is transformed by a specific phosphohydrolase into 1-O-alkyl-2-acetyl-sn-glycerol. The later is then converted to PAF by a dithiothreitol-insensitive CDP-cholinephosphotransferase. This pathway is mainly involved in the constitutive synthesis of PAF and is regulated only by the availability of substrates.
IV. PLATELET-ACTIVATING FACTOR RECEPTORS AND SIGNAL TRANSDUCTION

PAF acts via specific receptors on the membranes of responsive cells (38, 374) (Fig. 3). Binding studies revealed two distinct types of binding sites on human platelets (439). One binding site for PAF exhibited high affinity with a dissociation constant ($K_\text{d}$) value of 37 ± 13 nM and had a low capacity of 1,399 ± 498 sites/platelet. The second binding site showed nearly infinite binding capacity with a low affinity for PAF. The activation of platelets was due to the interaction of PAF with the high-affinity binding sites. Rabbit platelets showed, as human platelets, high-affinity binding sites for PAF with a $K_\text{d}$ of 0.9 ± 0.5 nM (184, 372, 435). In contrast, rat platelets that are insensitive to PAF action exhibited only the low-affinity binding site for PAF (196, 313). It was subsequently shown that specific binding sites for PAF are present in smooth muscle cells (193), cardiomyocytes (403), neutrophils (321, 441), monocytes-macrophages (257, 438), eosinophils (436), endothelial cells (223), and Kupffer cells (94, 95, 97). Moreover, PAF specific binding sites were identified in cells of the central nervous system. Three distinct classes of PAF binding sites have been detected in synaptic plasma membranes and intracellular membranes of rat cerebral cortex (121, 266, 416). Recently, it has been shown that PAF receptor in endothelial cells is expressed not only on the cell surface but also in the large endosomal compartment (195). The significance of intracellular receptors has not yet been clarified. However, it has been suggested that intracellular PAF receptors may mediate a PAF-dependent signal transduction pathway, as postulated for PAF-induced protooncogene expression (416). A cDNA for a PAF receptor from guinea pig lung has been cloned (185). The strategy used involved the construction of a cDNA library from side-fractionated poly(A) RNA, the synthesis of a transcript of the cDNA using phage DNA as template, and the expression of the transcript in the Xenopus oocytes (185). The analysis of PAF receptor cDNA indicated that PAF receptor belongs to the family of “serpentine receptors,” which contain seven $\alpha$-helical domains that wave in and out of the plasma membrane seven times. Surprisingly, PAF receptor contains only 342 amino acids and has a molecular mass of 38,982 Da (185). The third intracellular loop and the carboxyl tail, which is thought to bind G proteins in the serpentine receptor family, are very short in PAF receptor. It has been also found that there are nine potential phosphorylation sites on the carboxyl end of the receptor. Phosphorylation of the sites may modulate the binding of G proteins to the receptors and may account for the rapid desensitization of PAF receptors (310). Subsequently, PAF receptor was cloned from human leukocytes (232, 310) and HL-60 granulocytes (470). PAF receptor cloned from human leukocytes revealed 83% identity in the amino acid sequence with that of guinea pig lung. Recently, it has been shown that the PAF receptor protein expressed by human cardiomyocytes is exactly the same as that of human leukocytes (403). However, the 5’-non-coding region of cDNA encoding for cardiac PAF receptor is different from that of leukocytes, suggesting the presence of a tissue-specific regulatory mechanism (403). The induction of PAF receptor expression in Xenopus laevis oocytes and in COS-7 cells shows that PAF receptor is functionally linked to phosphoinositide metabolism by a G protein (310). Although researchers believe that PAF is

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**FIG. 3.** Schematic representation of PAF receptor-signaling mechanisms. PLA$_2$, phospholipase A$_2$; G protein, GTP-binding protein; PLC, phospholipase C; PtdInsP$_2$, phosphatidylinositol 4,5-bis-phosphate; inositol-P$_2$, inositol 4,5-bis-phosphate; InsP$_3$, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C.
coupled to PLC and PLA2 through a G protein, the G protein involved has not yet been fully characterized. It has been suggested that PAF binds to receptor and activates the associated G protein by exchanging guanosine triphosphate for guanosine diphosphate. In turn, the G protein activates a phosphatidylinositol-specific PLC (100). Therefore, stimulation of PAF receptors leads to the transient production of diacylglycerol, which activates PKC, and of inositol trisphosphate, which mediates the release of internal calcium stores. It has been proposed that the basic components of this pathway operate in all cells bearing PAF receptors and that the different responses to PAF depend on the function of target cells. Moreover, PAF has been found to stimulate the release of arachidonic acid in various cell types by different mechanisms (309, 311, 334, 449, 472). For instance, in neutrophils, PAF induces the activation of PLA2 by a mechanism requiring only the mobilization of intracellular calcium stores while in Kupffer cells PLA2 activation is dependent on extracellular calcium influx (95). In addition, the activation of PLA2 by PAF occurs through a PKC-dependent mechanism (96, 277, 320) and is prevented by pertussis toxin, suggesting a G protein involvement (311, 365, 411). PAF-induced activation of PLA2 is also regulated by the intracellular levels of cAMP (88, 96). The arachidonic acid metabolites have been shown to mediate several biological activities of PAF. In the heart, PAF-induced coronary vasoconstriction and reduced contractility are affected both by cyclooxygenase- and lipooxygenase-derived arachidonic metabolites (334, 335). Furthermore, PAF induces an elevation of cytosolic free calcium in several cell types including vascular smooth muscle cells (241, 369). The two main mechanisms involved in PAF-induced increase in cytosolic free calcium are 1) the mobilization of calcium from the intracellular stores as a result of inositol trisphosphate generation and 2) the influx of extracellular calcium through a membrane-associated channel regulated either directly by PAF or indirectly by intracellular second messenger such as lipooxygenase-derived metabolites of arachidonic acid (100). Calmodulin inhibition (254) and calcium channels antagonists, such as verapamil (105), were shown to block the influx of 45Ca2+ in rabbit platelets; moreover, verapamil prevents several in vitro and in vivo biological effects of PAF (12). Recently, it has been shown that PAF stimulates tyrosine phosphorylation of several proteins in platelets (117), neutrophils (153), and macrophages (98, 99). Moreover, it was found that PAF is capable of inducing the stimulation of NFkB activation (469) and the transcription of c-fos and c-jun genes in inflammatory cells (367). Because the PAF receptor contains several tyrosine residues in its intracellular loops and tail, it was suggested that tyrosine phosphorylation may be involved in the downregulation of the receptor (99). Recently, it has been shown that PAF may activate a mitogen-activated protein kinase (MAPK) (26, 375) and may induce the early tyrosine phosphorylation of focal adhesion kinase (p125FARK) in human endothelial cells (387). Moreover, in human neutrophils, PAF activates MAPK kinase-3, a known activator of p38 MAPK (315).

Many antagonists of the PAF receptor have been described including compounds without any structural relationship with PAF and structural analogs (for review, see Refs. 49, 86, 87, 166, 218, 362, 442). The development of potent and selective PAF receptor antagonists has been particularly valuable for studies on the pathophysiology of PAF (136). A number of natural PAF antagonists have been identified, including BN 52021 and kadsurenone, isolated from Ginkgo biloba and Piper futokadsura, respectively. More recently, several synthetic PAF receptor antagonists were also developed. They include 1) phospholipid analogs such as FR 72112, CV 3988, CV 6209, SRI 63441, ONO 6240, and RP 48740; 2) tetrahydrofuran derivatives such as L 652731; and 3) triazolobenzodiazepine derivatives such as WEB 2086, WEB 2170, BN 50726, and BN 50739. These chemically different PAF receptor antagonists share the ability to inhibit PAF binding to its receptor and to antagonize specific PAF-induced responses on target cells.

V. CARDIOVASCULAR RESPONSES TO PLATELET-ACTIVATING FACTOR

A. Hemodynamic Effect of PAF

The potential regulatory role of PAF on hemodynamics has been extensively studied. This line of research was initiated by the observation that one of the two antihypertensive lipids isolated from renal medulla and from renal venous effluent after unclipping one-kidney, one-clip hypertensive rats, the polar renomedullary lipid, has the same chemical structure of PAF (303, 304, 338). Early hemodynamic studies emphasized that antihypertensive polar renal lipid as well as synthetic PAF, in microgram doses, lowered arterial pressure in guinea pigs (135), rats (44, 67, 148, 233, 273, 344, 347, 360, 406, 430), and rabbits (12, 160, 161, 245, 290, 303) in the normal and hypertensive states after intravenous or oral administrations. The potential role of PAF in the modulation of blood pressure was inferred from the observation of decreased levels of PAF (275) and increased activity of plasma acetyl hydrolase (42) in hypertensive rats. Indeed, when administered intravenously, PAF is hypotensive in all species studied (134). Despite sensitivity variation among the species, several characteristics of hemodynamic response to PAF are common: the extent of hypotension is dose dependent, the onset is very rapid, the maximum effect is reached within 30–60 s, and the recovery time is also dose dependent. The basal mean arterial pressure (MAP)
is obtained within 5–10 min after intravenous injection. In animal species such as the rat, where platelets are insensitive to PAF action, no tachyphylaxis was observed (92, 120). In contrast, in the rabbit, where platelets are highly sensitive to PAF action, tachyphylaxis is present, suggesting that physiological alterations induced by PAF may at least in part occur via platelet activation and secretion of other mediators (160). Therefore, the rat model was used to differentiate direct vascular effects of PAF from those dependent on the activation of circulating platelets. Table 1 shows hemodynamic alterations induced by PAF infusion in different animal species. Although the mechanisms of PAF-induced hypotension are not completely understood, some data indicate that the action of PAF on the heart, peripheral vasculature, and microcirculation may account, at least in part, for the reduction of systemic blood pressure. PAF decreases the cardiac output; S, stable; I/D, increase followed by decrease; D/I, decrease followed by increase; ND, not determined.

TABLE 1. Effects of PAF on hemodynamic parameters in different animal species

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>MAP</th>
<th>HR</th>
<th>CO</th>
<th>TPR</th>
<th>MPAP</th>
<th>PVR</th>
<th>Reference No.</th>
</tr>
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<tr>
<td>Rat</td>
<td>0.3–1</td>
<td>D</td>
<td>I</td>
<td>D</td>
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<td>ND</td>
<td>ND</td>
<td>380</td>
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<tr>
<td></td>
<td>0.2</td>
<td>D</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>92</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>0.38</td>
<td>D</td>
<td>D</td>
<td>ND</td>
<td>ND</td>
<td>I</td>
<td>ND</td>
<td>160, 161</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.14–1.52</td>
<td>D</td>
<td>D</td>
<td>ND</td>
<td>ND</td>
<td>I</td>
<td>ND</td>
<td>290</td>
</tr>
<tr>
<td>Dog</td>
<td>0.038</td>
<td>D</td>
<td>S</td>
<td>I</td>
<td>D</td>
<td>ND</td>
<td>ND</td>
<td>468</td>
</tr>
<tr>
<td></td>
<td>0.19–0.35</td>
<td>D</td>
<td>ND</td>
<td>I/D</td>
<td>D/I</td>
<td>I/D</td>
<td>I</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>8–36</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>I</td>
<td>I/D</td>
<td>I</td>
<td>35</td>
</tr>
<tr>
<td>Pig</td>
<td>0.04–0.28</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>236</td>
</tr>
</tbody>
</table>

Doses are expressed as nmol platelet-activating factor (PAF)/kg intravenous bolus injection. MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; TPR, total peripheral resistance; MPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistances; I, increase; D, decrease; S, stable; I/D, increase followed by decrease; D/I, decrease followed by increase; ND, not determined.
onists markedly prevented the development of the second phase, namely, the rise in cardiac frequency, LVSP, MAP, and TPR, but did not significantly modify the first and third phases (290). Moreover, the generation of thromboxane A$_2$ from platelets may contribute to the development of hemodynamic alterations in rabbit. Indomethacin indeed determined an overall reduction in the extent of PAF-induced hemodynamic changes (290). These results suggest that the release of histamine and thromboxane A$_2$ from platelets may in part account for hemodynamic alterations induced by PAF. This was confirmed by the evidence of entrapment of platelets and leukocytes, particularly in the pulmonary microvasculature (279), and by experiments of platelet depletion (161). A calcium channel blocker, verapamil, was found to prevent all the hemodynamic and electric alterations induced by PAF (12). Administration of chronic intravenous PAF induces pulmonary arterial atrophy and hypertension with persistent increase in pulmonary resistances and reduction in cardiac output (323).

When the effects of PAF infusion were studied in the anesthetized dogs, a triphasic response was observed (35, 208). In the first phase (15–30 s), hypotension was attributed to a decrease in systemic vascular resistances that was associated with a rise in cardiac output. The second phase (30–90 s) consisted of sustained hypotension caused by a reduction in cardiac output associated with an increase in pulmonary and systemic resistances. The third phase was characterized by a gradual recovery of MAP, associated with a sevenfold rise in systemic vascular resistances and a persistent low cardiac output. Blockade of leukotriene receptors substantially inhibited the rise in systemic vascular resistances in the third phase, suggesting the role of leukotrienes as secondary mediators (208). The vasodilation observed in the first phase was independent from prostaglandin generation (208, 468). In contrast, the reduction of cardiac output observed in the second phase was shown to depend on generation of cyclooxygenase metabolites of arachidonic acid (468). Low doses of PAF caused only the first vasodilatory phase with hypotension (468). Studies performed in domestic pigs demonstrate an early pulmonary vasoconstriction with a right ventricular failure as the first determinant of PAF-induced shock. The subsequent decline in cardiac output, underfilling of the left ventricle, and systemic hypotension were interpreted as the consequence of right-sided events (149, 235, 236).

B. Effect of Local Administration of PAF on Selected Vascular Districts

In vivo the effects of PAF on selected blood vessels are masked by the sympathetic vasoconstrictor reflex and by mediators released from platelets and leukocytes. The effects of PAF depend on the doses and animal species (23, 46, 101, 109, 128, 133, 138, 165, 199, 210, 235, 282, 327, 363, 405, 444, 451). In rats, systemic administration of picomolar amounts of PAF, unable to induce changes of MAP, reduce vascular resistance and increase blood flow in the hindquarter, the mesenteric vessels, and the kidney (211, 380). In contrast, nanomolar concentrations of PAF injected into abdominal aorta proximal to the superior mesenteric artery or into the carotid artery induce vaso-
constriction (Table 2) (108, 148, 217). The injection of PAF into renal artery determines the following dose-dependent alterations: vasodilatation at 1 pmol/kg (165); initial vasodilatation followed by vasoconstriction at 4–19 pmol/kg (165), and vasoconstriction at 30–90 pmol/kg (23). In dogs, the prominent effect of intrarenal administration of PAF is vasoconstriction for all tested concentrations (24, 363). In contrast, in this animal species, PAF produces vasodilatation in gastric, mesenteric, and femoral arterial circulation (101). The vasodilator effect on femoral but not gastric and mesenteric vascular districts was dependent on prostaglandin synthesis. Moreover, the block of prostaglandin synthesis enhanced the vasoconstrictor effect of PAF on the renal vascular district (101).

When selected organs are perfused under constant pressure with low doses of PAF, where blood and autonomic nervous system control were absent, the common response was vasodilation. In these experimental conditions, PAF induces vasodilation in the kidney (368), but still produces vasoconstriction in the isolated lung (162, 175) and heart (30, 251, 335).

The question arises whether these experiments reflect only a pharmacological effect or a pathophysiological or a physiological role of PAF. It is hard to answer this question since it is difficult to measure the actual concentration of PAF in relevant fluids, cells, and tissues because this mediator is readily metabolized. The concentration of PAF in cells or tissues depends on the balance between its synthesis and degradation. The levels of PAF detected in blood and/or tissues in pathological conditions are in the range of nanograms and are therefore consistent with the doses used in experiments of exogenous administration. Therefore, it is conceivable that PAF may mediate hemodynamic changes occurring in pathophysiological conditions such as anaphylaxis and endotoxic shock or acute and chronic inflammation. Pharmacological agents that antagonize the binding of PAF to its receptors have been used to support this contention when it was found that they attenuate or reverse certain pathological processes. It is more difficult to evaluate a modulatory role of PAF on blood pressure in physiological conditions. The fact that PAF can act as vasoconstrictor at very low concentrations supports the hypothesis that endogenous PAF may be a regulator of blood pressure. The levels of PAF in the blood of normal subjects are in the range of picograms per milliliter (85, 359). In early studies it has been shown that PAF was not detectable in anephric hypertensive patients, suggesting a role of PAF synthesized in renal medulla in the regulation of blood pressure (85). This contention was not supported by subsequent studies, showing that the mean circulating PAF levels in patients with essential hypertension were not significantly different from those in normotensive subjects (359). However, it was found that high salt intake significantly increased the circulating levels of PAF, suggesting the synthesis of PAF to counteract the hypertensive effect of high dietary salt intake (359). Recently, it has been shown that there is an enhanced intracellular PAF-triggered signal transduction in approximately one-third of immortalized lymphoblasts derived from patients with essential hypertension (157). Moreover, it has been reported that PAF-acetylhydrolase activity in maternal and umbilical venous plasma was significantly lower in normotensive pregnant women than in nonpregnant women or in pregnancy-induced hypertension (215). This finding suggests that the inactivation of PAF by acetylhydrolase is decreased during normal pregnancy. Such modulation apparently does not occur in pregnant women that developed hypertension, suggesting that the catabolism of PAF plays a relevant role in the regulation of blood pressure in this contest (215).

TABLE 2. Effects of PAF on blood flow in selected vascular districts

<table>
<thead>
<tr>
<th>Species</th>
<th>Vascular District</th>
<th>Blood Flow</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Renal</td>
<td>0.001 nM</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.004–0.019 nM</td>
<td>I/D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02–0.1 nM/min</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03 nM/min</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>Cerebral</td>
<td>0.2–2 nM/min</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>Mesenteric</td>
<td>7.6 nM</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>Femoral</td>
<td>0.01–0.38 nM/min</td>
<td>D</td>
</tr>
<tr>
<td>Dog</td>
<td>Renal</td>
<td>0.04–0.19 nM/min</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Mesenteric</td>
<td>0.04–0.19 nM/min</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Coronary</td>
<td>0.5–2 nM</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Coronary</td>
<td>0.57 nM/min</td>
<td>I</td>
</tr>
<tr>
<td>Pig</td>
<td>Coronary</td>
<td>0.61–610 nM</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.38–3 nM</td>
<td>I/D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03 nM/min</td>
<td>I/D</td>
</tr>
</tbody>
</table>

I, increase; D, decrease; S, stable; I/D, increase followed by decrease; ND, not determined.

VI. EFFECT OF PLATELET-ACTIVATING FACTOR ON THE HEART

A. Coronary Circulation: In Vivo Effects

PAF administration into the coronary circulation induced variations in the coronary vascular tone depending on the doses and the animal species used. In the pig, intracoronary bolus injection of PAF produced a transient dose-dependent increase (up to 50% ED50 = 0.38 nM) in CBF followed by a second phase characterized by decrease (up to 92%) in CBF (ED50 = 0.92 nM) (133). In this study it was shown that at low doses of PAF (0.03–0.3 nM), both the increment and the decrement of CBF was present in the absence of significant changes in systemic
blood pressure. In contrast, the reduction in CBF caused by high doses of PAF was accompanied by significant decrease in systemic blood pressure and by electrocardiogram signs of ischemia such as S-T segment elevation or depression when flow decreases by more than 75% of control values. Similar results were reported after continuous intracoronary infusion of PAF in the pig (128, 134). Pharmacological studies demonstrated that the early increase in CBF is independent from the generation of cyclo- and lipoxygenase-derived metabolites, while the subsequent vasoconstriction is primarily due to the production of thromboxane A2 (133). PAF induces coronary vasoconstriction and S-T depression also in rabbits (290). Conflicting effects were observed in dogs (199, 282, 405). In one study it was shown that intracoronary injection of PAF reduced CBF concomitantly with a marked and rapid reduction in systemic arterial pressure and a negative inotropic response, effects that could have obscured the direct action of PAF on coronary artery (405). In other studies, similar doses of PAF are reported to produce a platelet-dependent coronary vasodilation (199) or a biphasic vasodilator/vasoconstrictor effect (282). In subsequent studies, it was found that PAF is vasodilator when the endothelium of coronary arteries is intact, whereas it induces vasoconstriction when the endothelium is injured as it may occur after ischemia (210). In vivo studies provided evidence that PAF induced a significant attenuation of endothelium-dependent dilation to intracoronary infusion of acetylcholine and serotonin, further suggesting an endothelial effect of PAF (111).

B. In Vitro Effects on Isolated Perfused Heart

In the isolated guinea pig heart perfused at constant pressure, PAF produced a dose-dependent increase in coronary vascular resistances (30, 149, 208, 251, 335, 398). This vasoconstrictor effect of PAF was completely blocked by verapamil, a calcium antagonist (12). Pharmacological inhibition of cyclooxygenase and blockade of leukotrienes receptors were reported ineffective by Levi et al. (251) and Stahl et al. (398), while PAF receptor antagonists reduce the coronary vasoactive effect of PAF (335, 410, 447, 448). Similar coronary vasoconstriction was obtained with high doses of PAF in isolated perfused rat heart (334, 397). However, when low doses were used, vasodilation alone or vasodilation followed by vasoconstriction was observed (187, 188). Pharmacological studies on the action of PAF on isolated rat heart suggest that both prostaglandins and leukotrienes are involved in the vasoconstrictor effect of PAF. However, it has been shown that lipoxygenase products are mainly responsible for the vasodilator and vasoconstrictor effects of PAF on coronary vasculature, whereas cyclooxygenase products play only a partial role (147, 265, 334, 397). In the isolated perfused rabbit heart, the coronary vasculature was apparently insensitive to PAF (208, 283, 291). However, when isolated rabbit heart was perfused with blood, PAF markedly reduced coronary flow (208). The isolated rabbit heart was used as a model to study in vitro interaction between platelets, leukocytes, and endothelial cells in the coronary circulation. In the rabbit heart perfused with platelets, the infusion of PAF induced a dose-dependent decrease of coronary flow, which was prevented by pretreatment of the heart with H1 and H2 histamine receptor antagonists and a leukotriene receptor antagonist (291). A similar protective effect was obtained after treatment of platelets with prostacyclin, which inhibits the activation of platelets by PAF (8). Perfusion of rabbit heart with PMN followed by PAF stimulation did not alter coronary tone (10). However, PMN may influence the effect of PAF on coronary vessels as a result of cooperation with platelets. The reduction of coronary flow induced by PAF in rabbit heart perfused with platelets and PMN was completely blocked by a leukotriene receptor antagonist, suggesting that leukotrienes released by PMN as a consequence of a PAF-induced cooperation with platelets are the main mediators of coronary constriction (10). This evidence is also supported by experiments of perfusion of rabbit heart with N-formyl-L-methionyl-L-leucyl-L-phenylalanine-activated PMN (417). In this experimental condition, PAF antagonist inhibited the neutrophil-dependent increase in coronary resistances (417). Similar results were obtained in studies on the PMN-induced contraction of the isolated rings of cat coronary artery (307). In humans, in basal conditions isolated coronary artery rings did not react to PAF challenge. However, Soloviev and Brachet (388) have shown that isolated human coronary artery strips after hypoxia undergo to a PAF-dependent biphasic contraction: an initial short phase of contraction, followed by a longer tonic shortening inhibited by treatment with a PAF receptor antagonist.

C. Myocardial Function

The alterations in cardiac function, including the reduction in cardiac output observed in vivo after infusion of PAF, can result either from a direct action on the heart or from indirect effects such as systemic changes and variations in pre- and afterload pressures. Furthermore, alterations in cardiac performance may depend on the effect of PAF on the coronary circulation, on the conduct system, and on the contractile properties of myocardium (150).

A direct effect of PAF on cardiac contractility was suggested by experiments of intracoronary infusion of low doses of PAF in pig, that induced a marked reduction in cardiac output as well as in regional shortening fraction in the absence of significant effects on systemic blood
pressure (126, 128, 133, 134). In vitro experiments on the isolated coronary perfused heart, the effect of PAF is quite different, depending on the animal species studied. In guinea pig isolated heart perfused at constant pressure, infusion of PAF reduces the force of contraction and the coronary flow in a dose-dependent manner. Moreover, PAF markedly alters the electrical activity of the heart (351); the severity and duration of these alterations are related to PAF dosage. PAF induces conduction arrhythmias, ranging from second degree atrioventricular conduction block to complete atrioventricular dissociation and ventricular extrasystoles, disappearance of the T wave, and depression of the S-T segment, a sign of myocardial ischemia (12, 30, 253, 357, 398, 410). The studies of ventricular action potentials by means of intracellular electrodes have shown that after the infusion of PAF the action potential duration progressively shortens, and the resting membrane potential, the overshoot, and the maximum rate of depolarization, which are initially unchanged, progressively reduce 10 min after the challenge (12). The response to PAF is prompt and long lasting; the maximal effect is reached within 2 min after the challenge. At 10 nM, PAF causes irreversible conduction arrhythmias so that the normal sinus rhythm and cardiac contractility do not return even after a prolonged perfusion with physiological solution without PAF. These effects seem to be independent from the release of acetylcholine from the nerve endings, since pretreatment with atropine does not affect the action of PAF (30). Moreover, the effects of PAF are independent from the generation of secondary mediators derived from the arachidonate metabolism, since neither the cyclooxygenase inhibitor indomethacin, nor the thromboxane synthetase inhibitors, nor the leukotriene receptor antagonists significantly modify the response to PAF (251, 253). In these experiments, peptide leukotrienes or thromboxane B₂ were not detected by radioimmunoassay in the coronary effluent (398). However, pretreatment of the heart with the calcium antagonist verapamil significantly reduces the entity of PAF-induced coronary spasm and completely abrogates both the electrical and mechanical alterations (12). The entity of cardiac alterations induced by PAF is related to the molecular structure for different species of PAF. In particular, the most potent species of PAF in inducing coronary constriction in the guinea pig heart was 18:1 PAF, whereas 16:0 and 18:0 had minor effects. The order of potency in reducing contractile force was 16:0 > 18:1 > 18:0. Therefore, the presence of a single double bond in the alkyl chain at the sn-1 position markedly alters the cardiac activity of alkyl-PAF (252, 280). In contrast to PAF, its deacetylated derivative lyso-PAF causes no significant variation in contractile force, coronary flow, and cardiac rhythm (251). The effects of PAF are also present in the isolated heart perfused at constant flow; in this case, a dose-dependent increase in coronary resistance is observed (251). Because a reduction of cardiac inotropism and alteration of cardiac rhythm induced by an intracoronary infusion of PAF are also present when the guinea pig isolated heart is perfused at constant pressure, it is unlikely that the effects of PAF depend on an ischemic state consequent to a reduction of coronary flow.

The effects induced by PAF in the isolated rat heart are similar to that observed in guinea pig heart (e.g., reduction in coronary flow and developed pressure, increase in diastolic pressure and conduction arrhythmias) (188, 189, 334, 339, 340). In this animal, however, it has been shown that the PAF-induced coronary vasoconstriction is mediated by the peptide leukotrienes (leukotriene C₄ and leukotriene D₄) (188, 189, 334). This finding indicates that in the rat heart PAF may have a small direct negative effect, whereas the reduction of coronary flow consequent to production and release of endogenous leukotrienes appears to induce a major depression of cardiac contractile force. Arachidonic acid metabolites released by PAF may stimulate the release of atrial natriuretic factor in rat heart (348, 349). Interestingly, comparison of the effect of PAF in adult and senescent rats shows that all the electrical and mechanical alterations induced by PAF are more marked in the senescent hearts (1). Isolated rabbit heart is responsive to PAF only if blood (208), platelets, and platelets plus leukocytes are present in the coronary vessels (283, 291). In these conditions, PAF induces mechanical and electrical alterations similar to those observed in the isolated guinea pig heart.

D. Atrium and Papillary Muscle

The isolated perfused heart preparation does not differentiate the direct negative inotropic effect of PAF from that consequent to changes in coronary flow and O₂ delivery to the heart. Therefore, the isolated atrium and papillary muscles were used to address the direct effect of PAF on cardiac muscle. In isolated rat atrium, PAF does not elicit significant effects on chronotropy and inotropy at concentration up to 3 × 10⁻⁵ M (89). At the concentration 1 × 10⁻⁴ M, PAF induces positive chronotropic and inotropic effects on isolated spontaneously beating right and electrically driven left atria. Both effects were blocked by propranolol, whereas reserpine pretreatment antagonized only the chronotropic response. Studies on isolated guinea pig atrium and papillary muscle (71, 118, 251, 407) showed a direct negative inotropic effect of PAF. Lyso-PAF caused only minimal effect (~5%) on these preparations. We studied the effect of PAF on the electrical activity of guinea pig papillary muscle by means of intracellular electrodes (71). PAF at 2 × 10⁻¹⁰ M induces a biphasic effect with an increase of 20% of contractile force within 2–3 min after the challenge, followed
by a progressive decrease of developed tension, which was reduced by 50–60% with respect to control values. The positive inotropic effect was preceded by a slight augmentation of action potential duration (APD); subsequently, APD decreased concomitantly with the negative inotropic effect. Pretreatment with propranolol prevented the positive inotropic effect and increase of APD. Similar biphasic dose-dependent effect of PAF was observed in guinea pig papillary muscle by Tamargo et al. (407). Moreover, these authors showed that at high concentrations of PAF (10\(^{-9}\) to 10\(^{-7}\) M), reduction of APD was accompanied by increase of action potential amplitude, maximum rate of depolarization (MRD), and resting membrane potential (\(E_R\)) of the action potential. Short runs of spontaneous discharges of ventricular muscle fibers accompanied the electrophysiological effects of PAF at all concentrations tested. A similar biphasic dose-dependent effect was described by Gollasch et al. (152), Kecskemeti (206), and Kecskemeti and Braquet (207) in guinea pig auricles: 10\(^{-10}\) M PAF induces transient positive inotropic effect followed by a negative one; 10\(^{-7}\) M PAF induces negative inotropic effect accompanied by decrease in amplitude, duration, and MRD of the action potential without changes in \(E_R\). The effect of PAF was reversed after washout of PAF. The study on the slow action potential was performed in these preparations to obtain insight into the mechanism of PAF-induced negative inotropism and decrease of APD. However, the results of these studies are partially conflicting. For instance, we reported that PAF induces a transient positive inotropic effect and enhancement of the slow action potential, followed by a profound depression of both the electrical and mechanical activities, suggesting a biphasic effect of PAF, i.e., an initial stimulation followed by depression of slow calcium channels (71). In contrast, Tamargo et al. (407) and Robertson et al. (353) found that PAF induces only a dose-dependent increase in amplitude and MRD of slow action potentials. These discrepancies are probably due to the different methods used. In our study, slow action potentials were obtained by elevating both K\(^+\) and Ca\(^{2+}\) extracellular concentrations to 22 and 6 mM, respectively, to inactivate the fast Na\(^+\) current and increase the driving force for Ca\(^{2+}\), while in the case of Tamargo et al. (407) and Robertson et al. (353) papillary muscles were bathed in high-K\(^+\) solution with the addition of isoproterenol or histamine to obtain slow action potentials. These drugs are known to stimulate adenylate cyclase via specific receptors, to increase the intracellular levels of cAMP, and to activate protein kinase A, leading to phosphorylation not only of the Ca\(^{2+}\) channel protein but also of a large number of other substrates within the cell. It has been reported that after pretreatment of papillary muscle with isoproterenol, which per se has a positive inotropic effect, PAF further enhances action potential amplitude and duration, as well as the force of contraction, while the second phase (depression of action potential and contractility) is absent. These results are consistent with that observed in the isolated rat heart by Bensard et al. (28), that PAF depresses basal myocardial function and enhances the functional response to \(\beta\)-adrenergic stimulation. Finally, the direct demonstration of the involvement of calcium current comes from voltage-clamp experiments on frog and guinea pig atrial fibers, in which Gollasch et al. (152) showed that the negative inotropic effect of PAF is accompanied by a significant reduction of this current. The mechanical and electrical changes induced by PAF were studied in human cardiac preparations such as the isolated papillary muscle (11) and atrial tissue (352). Challenge of human papillary muscles excised from the left ventricle by open heart surgery with various doses of PAF (1 \times 10^{-10} to 1 \times 10^{-6} M) induced a biphasic dose-dependent effect, characterized by a transient positive effect on inotropism and APD, followed by a marked prolonged negative effect on force of contraction and APD. No changes in \(E_R\), overshoot, and MRD of the action potential were detected after PAF challenge (11). Robertson et al. (352) reported a similar dose-dependent negative inotropic effect of PAF in human atrial muscle. However, lyso-PAF has no effect on contractile activity in both cardiac preparations, even at concentration up to 100-fold greater than those of PAF. In human papillary muscle, propranolol blocked the transient inotropic effect of PAF, suggesting a stimulation of \(\beta\)-receptors by endogenous catecholamines; pretreatment with indomethacin did not modify the initial positive effect, but markedly reduced the negative effect of PAF (11). In contrast, in human atrial tissue, the effect of PAF was blocked by antagonists of PAF receptor, but not modified by atropine, indomethacin, and the leukotriene receptor antagonist FPL 55712, suggesting a direct effect of this mediator (352). Moreover, it was shown that the decrease of contractile force caused by PAF in guinea pig papillary muscle may depend on the reduction of intracellular sodium activity, which may affect sodium/calcium exchange, finally causing reduction of intracellular calcium and contractility (353).

### E. Effects on Cardiomyocytes

The direct effects of PAF on cardiac inotropism and chronotropism have been studied in isolated adult or cultured neonatal cardiomyocytes (112, 272). In this experimental condition, PAF was shown to decrease myocardial twitch tension and velocity of contraction and relaxation as well as to increase spontaneous beating frequency (272). Recently, it has been shown that PAF-induced negative inotropic effect correlated with a decrease in systolic intracellular calcium concentration
Parallel biochemical studies demonstrated that PAF stimulates phosphoinositide pathway, leading to accumulation of \[^{3}H\]inositol phosphate and activation of PKC in cardiomyocytes (102). Because it has been shown that blockade of PAF receptors prevented both mechanical and biochemical changes induced by PAF, the presence of PAF receptors on cardiomyocytes may be suggested. The PAF receptor gene in human cardiomyocytes (403) has been recently cloned and characterized. Moreover, the patch-clamp technique has been applied to the study of electrophysiological effects of PAF on isolated guinea pig ventricular and atrial cells. Single-channel studies on cell-attached patches have shown that PAF affects inwardly rectifying background potassium channels (I\(_{K1}\)) (450). PAF initially induces a flickering of the channel, followed by a gradual prolonged depression of the channel activity. Because these potassium channels have a prominent role in determining the resting potential and excitability of the cardiac cells, it has been suggested that the effect of PAF on I\(_{K1}\) may play a major role in the electrophysiological action of PAF in the heart. Moreover, PAF was shown to stimulate cardiac muscarinic potassium channels in isolated guinea pig atrial cells. The effect of PAF was prevented by specific PAF receptor antagonists, lipoxygenase and PLA\(_2\) inhibitors, but not by cyclooxygenase antagonists. The opening of the channel was shown to be dependent on the activation of a G protein (309). Similar results were obtained using isolated bullfrog atrial myocytes (346). In contrast, in human and chick ventricular myocytes, PAF was found to stimulate T- and L-type calcium currents in a dose-dependent manner, whereas no effect on fast sodium current or delayed outward potassium current was observed. The effect of PAF on calcium currents was receptor dependent, because it was inhibited by PAF receptor blockade with WEB 2170 (41). The different response to PAF of cardiomyocytes derived from atrium or ventricle suggests a functional differentiation of these cells.

Moreover, it as been shown that PAF induces secretion of atrial natriuretic peptide (103, 348, 349) and eicosanoids (27) in spontaneously beating neonatal rat cardiomyocytes.

VII. MICROVASCULAR EFFECT OF PLATELET-ACTIVATING FACTOR IN VIVO

The potent vasoactive and leukotactic properties of PAF were initially studied in the rat cremaster muscle and skin after infusion of colloidal carbon and local injection of PAF (191). PAF was shown to be 1,000–10,000 times more potent than histamine on molar basis in inducing vascular permeability (191). Ultrastructural studies demonstrated subendothelial carbon accumulation in the postcapillary venules. A concomitant leukocyte margination was observed. However, the vasoactive properties of PAF in the rat appeared to be neutrophil and platelet independent. A direct stimulation of venular and endothelial cells was suggested. The effect of PAF on microcirculation has been studied also on the hamster cheek pouch (89, 119). Vasoconstriction was the predominant vasomotor response to PAF. This biological effect was dependent on the dose of PAF and the size of the vessel and was mediated by PAF receptor interaction and production of thromboxane A\(_2\). In addition, PAF increases vascular permeability both by a direct mechanism and platelet- and leukocyte-mediated mechanisms (119). It was found that PKC activation is an in vivo biochemical pathway in the signal transduction of PAF-stimulated microvascular cellular responses, leading to increases in the transport of the macromolecules. Indeed, PKC inhibitors significantly blocked the increase in intravascular permeability (214). In contrast, they did not interfere with the PAF-induced arteriolar constriction (214). Studies on intestinal microvasculature have shown that PAF promotes the filtration of fluid and protein across intestinal capillaries in cats (226). These microvascular effects of PAF are mediated in part by adherent leukocytes (226, 227). In the guinea pig, PAF causes a dose-dependent increase in airway vascular permeability, as measured by extravasation of Evans blue dye, at concentrations as low as 1 ng/kg (168). The site of leakage, as for other mediators, is in the postcapillary venules. This effect of PAF is receptor mediated and platelet independent, as it is inhibited by receptor antagonists, but not by platelet depletion. PAF also affects mean renal vascular resistance (23) and glomerular permeability (82, 364) by a dual mechanism: 1) it enhances size permeability of glomerular basal membrane by a direct action on the glomerular capillary wall (330), and 2) it modifies perme-selectivity owing to a loss of fixed negative charges of the glomerular capillary wall due to the release of cationic proteins from PAF-activated platelets and neutrophils (82). Intradermal injection of PAF into the skin of human volunteers has been reported to produce an immediate vasoconstriction followed by a vasodilatation and an increase in vascular permeability (19). The increase in plasma protein extravasation elicited by PAF was enhanced by concomitant intradermal injections of prostaglandin E\(_2\) or prostaglandin E\(_1\) and inhibited by a \(\beta\)-adrenoceptor agonist (isoprenaline) or an \(\alpha\)-adrenoceptor agonist (phenylephrine) (300). The effect of PAF on vascular permeability was independent from the stimulation of H\(_1\) histamine receptor (191). Recently, it has been shown that the synthesis of PAF mediates the increase in vascular permeability induced by vascular endothelial growth factor (VEGF) in certain organs, such as stomach, duodenum, and pancreas (381), but not in skin (308).
VIII. EFFECTS OF PLATELET-ACTIVATING FACTOR ON ENDOTHELIAL CELLS

Endothelium modulates the microenvironment homeostasis by affecting the traffic of macromolecules and cells from the bloodstream to the tissue. The molecules involved in the control of such traffic are soluble mediators, surface receptors that translate external signals and adhesion molecules. In this setting, PAF acts as an autocrine (176) and paracrine mediator that may modulate endothelial functions. Several stimuli are capable of inducing the synthesis of PAF from endothelium including thrombin, vasoactive mediators, and proinflammatory cytokines, suggesting that PAF may transduce or amplify the signals delivered by these mediators. The two main endothelial cell functions regulated by the synthesis of PAF are the endothelial cell barrier function and the adhesion of leukocytes to the endothelial layer, which precedes their transmigration.

A. Effect of PAF on Endothelial Cell Permeability

The endothelium was shown to express PAF-binding sites (223) and to be a target for PAF (62). Therefore, PAF produced by endothelial or inflammatory cells may stimulate endothelial cell functions. In vitro PAF enhances the permeability of cultured human endothelial cell monolayer and induces changes of the cell cytoskeleton leading to cell retraction and formation of intercellular gaps (62). Specific PAF receptor antagonists (62) inhibit these effects. Shape change of bovine pulmonary endothelial cells was also obtained in the presence of PAF (57, 155). Moreover, PAF induces the production from endothelial cells of several vasoactive mediators. PAF stimulation of cultured human endothelial cells (117a) induces a dose-dependent synthesis of prostacyclin and thromboxane A2, or release of plasminogen activator (124). In addition, the changes in shape of endothelial cells were associated with activation of calcium-dependent K+ channels and hyperpolarization of cell membrane. PAF induced an increase in cytosolic free calcium through the production of inositol trisphosphate (41, 54, 58, 155), and possibly the opening of receptor-operated calcium channels may serve as a signal for increasing macromolecular transport and activation of PLA2. The stimulation of PLA2 may lead to the synthesis and release of leukotrienes and thromboxane A2, which are involved in PAF-induced permeability changes and arteriolar constriction, respectively. Recent studies on the basic mechanism by which leukocyte-endothelial cell adhesion mediates PAF-induced increases in capillary permeability demonstrate a correlation between the extent of arteriolar pairing to venules and the PAF-induced increase in capillary fluid filtration rate (170).

B. Endothelium-Leukocyte and -Platelet Interaction

Three subsequent steps are thought to be involved in neutrophil migration from bloodstream to inflamed tissues (66). The first step requires a transient interaction of leukocytes with the endothelial cells mediated by surface molecule known as selectins. This interaction induces the rolling of leukocytes along the vessel wall, but it is not strong enough to completely stop them. The second step involves the activation of the leukocytes brought, by selectins, into contact with endothelium. This activation leads to a stable adhesion dependent on the interaction of integrins expressed on the surface of leukocytes with the endothelial counter receptors belonging to the superfamilly immunoglobulins. In the third step, chemoattractants stimulate the transmigration of leukocytes across the vessel wall (390). PAF is considered a mediator of leukocyte-endothelium interaction, which may participate in the cell activation phase (479, 480). Prescott et al. (336) correlated the adhesion of neutrophils to thrombin-activated endothelium with the PAF synthesized and expressed on the surface of endothelial cells. By using specific PAF receptor antagonists, they demonstrated that PAF produced by stimulated endothelial cells was a crucial determinant in neutrophil adhesion to endothelium. PAF produced after thrombin stimulation is coexpressed with P-selectin on the endothelial cell surface. It has been suggested that P-selectin triggers and PAF activates neutrophils by interacting with its specific receptor (259, 376). This leads to an influx of calcium ions in neutrophils associated with the upfunctional regulation of CD11/CD18 integrin complex and the cell polarization (259). Moreover, PAF was shown to play a different role when endothelial cells are stimulated by cytokines such as IL-1 (61, 228, 229) or TNF-α (64, 228). Cytokines promoted the neosynthesis and coexpression on the endothelial cell surface of a selectin (E-selectin) (390) and of PAF. The latter promoted a calcium influx in adhering neutrophils (228, 229) that enhanced their response to the IL-8 produced by endothelial cells (228). PAF was not essential in the delayed cytokine-induced PMN adhesion (228, 229) but was involved in neutrophil emigration, because PAF receptor antagonists were shown to block the neutrophil migration across monolayers of cytokine-pretreated endothelial cells (228). The difference in the role of PAF exposed on endothelial cell surface after thrombin or cytokines treatment may depend either on differences on molecules coexpressed on the endothelial cell surface or on the amount of PAF exposed. Finally, exogenously added PAF increases the endothelial cell proadhesive properties for leukocytes (336, 376, 476, 477) and promotes their transendothelial migration (88). In this experimental condition, PAF stimulates the endothelial cell receptor, promoting the expression of P-selectin and a
rapid and selective loss of sulfated proteoglycans, thus reducing the charged repelling forces between cells (376). Indeed, it has been shown that PAF plays a critical role in monocytes as well as neutrophil migration across monolayers of cytokine-prestimulated endothelial cells (228). Recently, it has been shown that integrins are up-regulated by PAF and that the $\beta_1$-integrins are critically involved in PAF-induced leukocyte locomotion in extravascular tissue (454). Moreover, platelet/endothelial cell adhesion molecule-1 has been involved in PAF-induced cell activation, suggesting that platelet/endothelial cell adhesion molecule-1 may serve as a costimulatory agonist receptor capable of modulating integrin function in human platelets during adhesion and aggregation (443).

These mechanisms of leukocyte recruitment were shown to play a critical role in the ischemia-reperfusion injury (Fig. 5) (261). Indeed, it was found that cultured human endothelial cells exposed to sublethal anoxia followed by reoxygenation induced PAF synthesis (84) and a leukocyte adhesion and transmigration that was inhibited by PAF receptor antagonists (20, 194, 201, 284, 345, 471). It was found that cultured human endothelial cells and neonatal rat heart myocytes synthesize PAF after prolonged hypoxia (56). Moreover, several studies demonstrated that monoclonal antibodies against adhesion molecules as well as antagonists of selectins or of PAF significantly prevented the recruitment of leukocytes in ischemic heart and reduced the necrotic area (261, 287, 307).

The role of PAF as mediator of direct interaction between platelets and endothelium is controversial. In vitro studies have shown that exogenously added PAF did not promote platelet adhesion to an endothelial monolayer. However, it has been recently shown that PAF, but not leukotriene B4, induces the adhesion of platelets to the endothelium in the presence of activated PMN (180–182). The inhibitory effect of PAF receptor antagonists suggests that PAF mediate a PMN-platelet interaction. The generation of oxygen radicals from activated PMN was shown to stimulate the subsequent adhesion of platelets to the endothelium (182).

IX. INVOLVEMENT OF PLATELET-ACTIVATING FACTOR IN CARDIOVASCULAR PATHOPHYSIOLOGICAL PROCESSES

A. Role of PAF in Cardiac Anaphylaxis and Shock Syndromes

Cardiac anaphylaxis defines the involvement of the heart as a target organ of immediate hypersensitivity reaction. The in vitro studies on isolated guinea pig heart passively sensitized to dinitrophenol demonstrated a coronary vasoconstriction, impaired myocardial contractility, and arrhythmias following the antigen challenge (251). A hallmark of cardiac anaphylaxis is the release of mediators such as histamine, thromboxane A2, prostacyclin, and leukotrienes (5, 6, 251, 334). During anaphylaxis PAF is released in the coronary effluent of the isolated guinea pig heart (251). Moreover, when administered to isolated perfused heart, PAF reproduces the mechanical and electrical changes typically encountered during allergic reactions (e.g., rightward shifts in the QRS axis, ischemic S-T segment changes, and brady- and tachyarrhythmias) (30, 251, 334, 335). In vivo, an intravascular release of PAF occurs during experimentally induced anaphylaxis in rab-

![Image](http://physrev.physiology.org/)

**FIG. 5.** Role of PAF in leukocyte recruitment and transmigration in injured myocardium. P-selectin contained in the bodies of Weibel-Palade (WP) is coexpressed with PAF on the surface of endothelium activated by hypoxia/reoxygenation. P-selectin by recognizing its counterreceptor sialyl Lewis X (sLeX) allows a transient adhesion of leukocytes leading to interaction of leukocyte PAF receptor (PAF-Rc) with PAF expressed on the endothelial cell surface. PAF stimulates an upfunctional regulation of CD11/CD18 integrin complex, making it competent to bind specific endothelial ligands such as intercellular adhesion molecule-1 (ICAM-1), leading to a firm adhesion. Moreover, PAF acts in concert with interleukin-8 (IL-8) in promoting the transmigration of leukocytes in the extravascular space. LTB4, leukotriene B4; PMN, polymorphonuclear neutrophils; EC, endothelial cells.
bits and implies a relationship between PAF and other mediators (110, 132, 160, 161, 332). It has been shown that the action of PAF on isolated rat heart was dependent on the release of leukotrienes and of cyclooxygenase products with vasoconstrictor action (334). A partial protective effect of PAF antagonists in anaphylactic reactions further supports the role of PAF. BN 52021 and WEB 2086 were shown to inhibit cardiac anaphylactic responses such as coronary constriction and decreased contractility of passively sensitized guinea pig hearts (219, 264). Moreover, the PAF receptor antagonists were shown to reduce significantly the sustained vasoconstriction induced by challenge with ovalbumin of presensitized animals (220). Even though indomethacin suppresses the production of 6-keto-prostaglandin F1α and thromboxane B2, it did not affect vasoconstriction during anaphylaxis (401). These observations suggest that PAF-induced coronary constriction depends on leukotriene production rather than thromboxane A2. In addition, PAF has been involved in the pathogenesis of immune complex-induced and septic shock. PAF was implicated in the hemocoagulation and the systemic enhancement of vascular permeability induced by infusion of immune complexes or IgG aggregates (167, 361). In this experimental condition, PAF also mediates leukopenia and thrombocytopenia induced by the immune complexes (81). Moreover, PAF has been considered a mediator of septic shock (75, 78, 137) on the basis of the following evidence: 1) when administered in experimental animals PAF reproduces several aspects of the lipopolysaccharides (LPS)- or TNF-α-induced shock (70, 134, 306, 344, 412). 2) PAF is synthesized during septic shock (120) by several cell types including monocytes, PMN, Kupffer cells, splenic cells, and endothelial cells stimulated either by LPS (74, 159, 198, 238), bacterial exotoxins (55, 225), porins (432), or TNF-α (64, 73, 440). Recently, we demonstrated that LPS-binding protein and CD14 (463) modulate the synthesis of PAF induced by LPS (74). 3) Transgenic mice overexpressing PAF receptor show an increased mortality when exposed to bacterial endotoxin (197). 4) PAF receptor antagonists inhibit or reverse endotoxin/TNF-α-induced hypotension and reduce mortality (4, 86, 93, 120, 140, 166, 205, 324, 325, 343, 370, 406, 412, 422, 462, 465, 467, 473). It has been shown that a variety of chemically unrelated PAF antagonists inhibit and/or reverse also the endotoxin-induced leukopenia, thrombocytopenia, and hemocoagulation (4, 107, 234, 422). Moreover, PAF receptor blockade has been shown to improve cardiovascular function in nonhypotensive sepsis (2, 125). Further support for the role of PAF was provided by the observation that a PAF receptor antagonist attenuates the induction of the cytokine network in experimental endotoxemia in pigs (462) and chimpanzees (230). Therefore, it has been suggested that PAF is the most proximal mediator in the cytokine cascade triggered by endotoxin or sepsis (230). There is evidence suggesting that PAF contributes to the pathogenesis of cardiac, lung, and renal complications of septic shock (328). Several negative inotropic substances such as TNF-α and IL-1 are present in the circulation (231). Because PAF was shown to mediate several biological effects of TNF-α (68), it is therefore a potential candidate for mediation of cardiac function depression in septic shock (11, 13, 30, 178, 205). In the guinea pig papillary muscle, cardiac alterations induced by TNF-α are mediated by PAF and nitric oxide, the production of which is downstream to the synthesis of PAF (13). Recently, it has been shown that PAF mediates the action of LPS on coronary microcirculation in isolated perfused rat heart (83). Moreover, PAF antagonists were shown to prevent systemic and pulmonary hemodynamic changes as well as acute lung and renal vascular injury in endotoxin-treated rats (92, 328, 420, 451). In the kidney, the synthesis of PAF from glomerular mesangial cells and endothelial cells may be triggered either directly by LPS or other bacterial products such as porins or by LPS-induced cytokines such as TNF-α and IL-1 (36, 72, 432). PAF extracted from kidney is increased in endotoxic shock, and PAF receptor antagonists not only prevent but also revert the normotensive LPS-induced hemodynamic insufficiency in rats (420, 451). In humans, thrombocytopenia and reduction of PAF free receptors were observed in septic shock, and the involvement of platelets was correlated with development of adult respiratory distress syndrome (ARDS) (258, 274, 389). Moreover, PAF has been detected also in bronchoalveolar lavage of patients with ARDS (274). A preliminary trial in which a PAF receptor antagonist has been used in the treatment of patients with septic shock has been recently published. This study suggests that mortality is reduced in gram-negative but not in gram-positive septic shock (116).

Furthermore, studies on anesthetized rats support the role of PAF in mediating traumatic shock. An increased content of PAF in the peritoneal fluid of traumatized rats and a partial protection from shock of several PAF receptor antagonists has been reported (393, 395, 413). On the basis of experiments with PAF receptor antagonists, PAF has been also implicated in hemorrhagic shock (3, 271, 394) and in postischemic shock reaction (358), although no direct evidence is available on an enhanced synthesis of this mediator.

B. Role of PAF in Ischemia-Reperfusion Injury of the Heart

The role of PAF in ischemia and reperfusion injury of the heart is supported by experiments aimed at evaluating
the synthesis of PAF and the protective effect of PAF receptor antagonists in these physiopathological conditions (47). Myocardial synthesis of PAF occurs in baboons following myocardial infarction (17), and an intravascular release of this mediator was detected in blood of patients with coronary artery disease undergoing atrial pacing to evaluate the severity of ischemia (294). Moreover, PAF was detected in the coronary sinus after occlusion and reperfusion injury in sheep (212). Indirect evidence for PAF biosynthesis during myocardial ischemia was obtained measuring the lyso-PAF, a metabolite of PAF, in canine myocardium subjected to permanent ligation of the left anterior descending coronary branch (247). Experiments on isolated perfused hearts demonstrated the cardiac origin of PAF released in significant amounts during ischemic reperfusion injury. In rabbits, PAF was detected in the coronary effluent during the initial reperfusion of ischemic heart (34, 204, 291). The release of PAF was concomitant with that of 6-keto-prostaglandin F(1alpha), an enzyme product of arachidonate metabolism (34). The precise cellular source of PAF was not identified in this model; however, likely candidates are endothelial cells (69, 276, 278, 336, 478) and cardiomyocytes (56). Indeed, it has been reported that cultured endothelial cells (20, 471) as well as cultured neonatal rat heart myocytes synthesize PAF after prolonged hypoxia (56). In the isolated rabbit heart, the effects of PAF were platelet dependent (291). When reperfusion was performed in the presence of autologous platelets, there was a significant worsening of left ventricular function and an increased rate of ventricular arrhythmias, which were prevented by a PAF receptor antagonist (90). The effect of platelets was due to the release of histamine, thromboxane A(2), and leukotrienes (291). It was shown that a PAF-dependent PMN-platelet cooperation significantly worsens reperfusion injury (10).

The vasoactive effect of PAF and its PMN-dependent mechanism have been directly studied in coronary resistance vessels using an isolated and perfused microvessel preparation (190). In this study topical application of PAF to the vessels induced a dose-dependent decrease in the diameter but an increase in the apparent permeability coefficient of albumin. Disruption of the endothelium abolished the vasoconstrictor response to PAF, and perfusion of PMN significantly augmented PAF-induced changes in vasomotor tone and permeability. Furthermore, administration of PAF caused PMN adhesion to the endothelium of coronary arterioles at low-flow perfusion velocities. These results suggest that PAF induces vasoconstriction and hyperpermeability in coronary arterioles via an endothelium-dependent and PMN-mediated mechanism and that PAF is able to stimulate PMN adhesion in coronary arterioles under a condition of low flow rate (190). Moreover, a link exists between the well-established role of oxygen radicals and that of PAF in ischemia reperfusion injury. In fact, it was found that generation of oxygen radicals stimulates the synthesis of PAF by endothelium in isolated perfused guinea pig heart and that a PAF receptor antagonist blunts the mechanical and electrical alterations induced by oxygen radicals (9). Additional support for the role of PAF in myocardial ischemia was obtained in experiments of PAF administration after induction of cardiac ischemia (250, 283, 393). In this experimental condition, the infusion of PAF significantly enhanced the ischemic injury by a mechanism dependent on thromboxane A2 generation (283). Finally, the effect of PAF receptor antagonists was studied in four experimental conditions: (1) the isolated perfused heart under conditions of ischemia followed by reperfusion, (2) the experimental myocardial infarction by coronary occlusion and reperfusion, (3) in a model of low-flow high-demand ischemia and reperfusion (366), and (4) in the cyclic constriction of a coronary artery to mimic the clinical situation of unstable angina. In the isolated perfused heart, PAF antagonists prevented both the platelet-dependent and platelet-independent mechanical and electrical alterations that occurred, respectively, in rabbit (10, 34, 291), guinea pig (141), and rat heart (127, 221, 222) after ischemia-reperfusion. In the experimental myocardial infarction by coronary occlusion, PAF receptor antagonists reduced the hematological and hemodynamic alterations as well as the size of necrotic area and the accumulation of platelets and leukocytes observed in rabbit (287, 288) and sheep (213) hearts but did not affect plasma protein leakage (91, 287, 459). In rats, PAF antagonists reduced infarct size and arrhythmias (144, 158, 263, 265). In dogs, conflicting results were obtained with different PAF receptor antagonists. With the use of BN 52021, SRI 63441, and TCV-309, a protective effect was observed (156, 267–270, 408), whereas WEB 2086 was ineffective (248, 249). Moreover, it was found that CV 6209 prevented pulmonary edema following coronary ligation in dogs (409). With the use of PAF antagonists in the cyclic constriction of a coronary artery to mimic the clinical situation of unstable angina, it was found that PAF contribute to platelet activation involved in the cyclic flow variations at the site of arterial stenosis and endothelial injury (151). It was shown that the cyclic flow variations depend on the release of mediators from platelets (18). In humans, an increase of PAF concentration in blood was observed in patients with defined unstable angina (371). Moreover, acute myocardial infarction is associated with a depression of plasma acetyl hydrolase activity that may allow a prolonged half-life of newly synthesized PAF (316, 400). Platelets from patients with acute myocardial infarction exhibit an increased sensitivity to the aggregatory effect of PAF in vitro in the first 48 h after the onset of the symptoms (431). We (293) and Graham et al. (154) were unable to find an increased synthesis of PAF in peripheral blood of patients with myocardial infarction. Whether a local synthesis of PAF occurs is unknown. However, suc-
cessful thrombolytic therapy with streptokinase was associated with intravascular release of PAF. Streptokinase as well as plasmin were shown to stimulate PAF synthesis by endothelium, an event that may limit the beneficial effect of thrombolytic therapy by promoting platelet and leukocyte adhesion and activation on the endothelial cell surface as well as transmigration into ischemic tissue (289, 293, 298). Indeed, it has been shown that PAF receptor antagonists prevent thrombotic reocclusion in dogs treated with recombinant-tissue-type plasminogen activator (421). Moreover, blockade of PAF receptors abrogated hypotension and platelet activation in rabbits treated with streptokinase and recombinant-tissue-type plasminogen activator (289).

Recently, it has been reported that PAF released during angioplasty in humans (123) mediates the neutrophil stimulation seen in this clinical setting (377, 378).

C. Role of PAF in Atherogenesis

PAF may play a role in atherogenesis and atherosclerosis (183, 402). The possible involvement of PAF in cholesterol deposition in the arterial wall has been investigated in rabbits fed a hypercholesterolemic diet (131). The administration of a PAF receptor antagonist to these rabbits significantly reduced the amount of esterified cholesterol in the aorta without affecting the plasma levels of cholesterol (131). Clinical studies show higher levels of PAF in coronary artery samples from patients with severe atherosclerosis (302). It has been suggested that PAF synthesized by endothelial cells and exposed on the cell surface may, together with P selectin, promote leukocyte adhesion to endothelial cells (479, 480). This interaction may be important for the activation and the subsequent infiltration of monocytes-macrophages, for the production of proliferative cytokines, and eventually for the accumulation of lipids within the cells (355). It has been shown that PAF and P-selectin cooperate in the nuclear translocation of a transcription factor NFkB and in the secretion of NFkB-dependent cytokines by monocytes. PAF has a weak agonist effect for NFkB-dependent actions in nonadherent monocytes (456). In contrast, the adhesion to P-selectin expressed on the endothelial surface amplifies or integrates signals triggered by PAF receptor, leading to the activation of NFkB-dependent functions (224, 455, 456). Furthermore, PAF stimulates, in monocytes, transcription of a heparin binding epidermal growth factor, and in vascular smooth muscle, synthesis of IL-6 that may act as potent mitogen for vascular smooth muscle cells (146, 329). Moreover, it has been shown that PAF mediates at least in part the adhesion of monocytes to endothelium induced by LDL and oxidized LDL (246). Cigarette smoking, a factor associated with the pathogenesis of atherosclerosis, causes platelet activation, LDL oxidative changes, and increased levels of PAF (285). The latter alteration was associated with a compensatory increase of PAF-AH activity. However, in vitro studies demonstrated that cigarette-derived products as well as oxidative changes of LDL, that physiologically carry PAF specific acetyl hydrolase, inhibit the activity of the enzyme that catabolizes PAF (285). Furthermore, PAF may also oxidize LDL, via stimulation of human monocytes/macrophages and neutrophils to produce superoxide anions and hydrogen peroxide (356, 419). PAF may also induce a release of proteases such as elastase from leukocytes that may degrade components of the extracellular matrix of the intima (356). This may favor the fissuration of the plaque (256). Indeed, an enhanced concentration of PAF was detected in endarterectomy samples of patients with complicated coronary plaques (302). It has been also shown that PAF is transiently produced by macrophages and cholesterol-loaded macrophage foam cells activated by phagocytosis, suggesting that PAF of macrophage origin may exert potent proinflammatory, proatherogenic, and prothrombotic effects (114).

X. ROLE OF PLATELET-ACTIVATING FACTOR IN NEOANGIOGENESIS

Neoangiogenesis has an important role in the embryogenesis of the heart and in the repair of myocardial infarction. The angiogenic process is, in physiological conditions, highly regulated to direct the organ development or to limit the growth of new blood vessels to the repaired tissue. An unregulated growth of blood vessels may be involved in pathological processes such as chronic inflammation, rupture of coronary plaques and intraplaque hemorrhage, and growth of most solid tumors (142, 143). In these conditions, angiogenesis may contribute to the development of tissue injury. Several angiogenic factors have been shown to modulate angiogenesis (142, 143). Endothelial cells are the primary target for these mediators and are stimulated to degrade extracellular matrix, migrate, and proliferate. These events are required to initiate a capillary sprout and the formation of new vessels. In this complex process, endothelium is actively involved and is capable of producing autocrine mediators such as the vascular endothelial growth factor (142, 143) and IL-8 (216). The relevance of angiogenesis in the recovery from myocardial infarction is supported by the recent observations that the administration of basic fibroblast growth factor and heparin significantly improves the collateral formation (169, 237, 342, 433). There is evidence indicating that PAF may act as a mediator of angiogenesis (15, 16, 59, 76, 205). Whereas in vitro PAF has only a chemotactic effect on endothelial cells but does not stimulate endothelial cell proliferation (76), in
vivo PAF can induce an angiogenic response. The angiogenic effect of PAF is either a heparin-independent or heparin-dependent mechanism according to concentration (76). At micromolar concentrations, PAF induces an angiogenesis independent from addition of exogenous heparin, possibly because the inflammatory reaction elicited may determine heparin release from mastocytes and endothelial cells (15, 16). In contrast, at nanomolar concentrations, PAF requires the addition of exogenous heparin for its angiogenic activity, suggesting the production of heparin binding growth factors (59, 76). PAF may directly provide the signal for migration but not the signal for endothelial cell proliferation, which is induced by heparin-binding growth factors, produced by PAF-stimulated endothelial cells. Indeed, PAF induced the expression of several angiogenic factors and chemokines including acid and basic fibroblast growth factor, vascular endothelial growth factor and its specific receptor flk-1, hepatocyte growth factor, and macrophage inflammatory protein 2 (59, 475). Moreover, we recently observed that PAF-induced neoangiogenesis was dependent on the production of nitric oxide (297). In vivo and in vitro experiments, performed with a panel of different PAF receptor antagonists, suggest that the synthesis of PAF induced by several polypeptide mediators, such as TNF-α, hepatocyte growth factor, VEGF, thrombopoietin, and IL-3, accounts for the endothelial migration required for the development of the new vessels (53, 77, 115, 295, 296). In contrast, the neoangiogenic effect of basic fibroblast growth factor appears to be independent from the expression of the PAF bioactivity (297).

XI. CONCLUSIONS

Despite growing evidence indicating a role of PAF in several pathological conditions, this mediator is still in search of a defined physiological role. The main difficulties in studying the physiological functions of PAF are related to technical hindrance in dosing the mediator, which in normal cells and tissues is synthesized in picomolar concentrations. Significant progress was achieved after cloning the receptor and the main catabolic enzyme PAF-AH. Moreover, functional information was derived from the use of several chemically unrelated PAF receptor antagonists. The in vitro studies provided evidence for a role of PAF both as intercellular and intracellular messenger involved in cell-to-cell communication. Triggering of PAF receptors was shown to elicit different responses, depending on cell type, PAF concentration, and cooperation with other intercellular mediators or intracellular messengers. In the cardiovascular system, PAF may have a role in embryogenesis, because it possesses angiogenic properties and acts by amplifying the effect of defined polypeptide mediators. PAF has been also implicated in the physiological modulation of blood pressure, mainly by affecting the renal vascular circulation. However, most of the available studies have been performed using nanomolar concentration of the mediator, which are reached only in physiopathological conditions. In the cardiovascular system, PAF has been involved in the hypotension and cardiac dysfunctions occurring in cardiac anaphylaxis and in various cardiovascular stress situations such as septic, hemorrhagic, and traumatic shocks. Moreover, PAF cooperate in the recruitment of leukocytes in inflamed tissue, promoting the activation of cells ensuing in adhesion to the endothelium and extravascular transmigration of leukocytes. The autocrine and paracrine effects of PAF are also involved in the enhancement of endothelial cell permeability and regulation of macro- and microvascular tone. Moreover, the angiogenic properties of PAF may contribute either to the development of chronic inflammatory angiogenesis or to restoration of the collateral blood flow in ischemic tissue. The finding that PAF is present in complicated atherosclerotic plaques, where neoangiogenesis has been implicated in the fissuration, suggests that PAF may have a role in the evolution of atherosclerotic lesion. Finally, studies based on measurement of the local production of PAF and on the action of PAF receptor antagonists have indicated that this mediator is critical in the development of myocardial ischemia-reperfusion injury and of adverse effects of thrombolytic therapy. In particular, the finding that the human heart can produce PAF, expresses PAF receptor, and is sensitive to the negative inotropic action of PAF suggests that this mediator may have a role in a local response of the heart to injury.

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