Platelet-Activating Factor Receptor

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Platelet-activating factor (PAF) is a pro-inflammatory lipid mediator possessing a unique 1-*O*-alkyl glycerophospholipid (GPC) backbone (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholin). Cloned PAF receptor, which belongs to the G protein–coupled receptor superfamily, transduces pleiotropic functions including cell motility, smooth muscle contraction, and synthesis and release of mediators and cytokines *via* multiple hetero-trimeric G proteins. Pharmacological studies have suggested that PAF functions in a variety of settings including allergy, inflammation, neural functions, reproduction, and atherosclerosis. Establishment of PAFR$^{-/-}$ mice confirmed that the PAF receptor is responsible for pro-inflammatory responses, but that its roles in other settings remain to be clarified.

Key words: bronchial asthma, endotoxin shock, G protein–coupled receptors, oxidized phospholipids, platelet-activating factor.

Overview

Platelet-activating factor (PAF), a structurally unusual lipid autacoid possessing an intact 1-*O*-alkyl glycerophospholipid (GPC) backbone (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholin), was originally identified as a pro-inflammatory mediator in the late 1970s. Subsequent researches suggest that PAF, and structurally related GPC oxidatively fragmented at the *sn*-2 position, function as mediators in a variety of settings including atherosclelosis, neural functions and reproduction. Cloned PAF receptor (PAFR) possesses a typical structure of G protein–coupled receptors (GPCRs) with seven transmembrane helices, and it presumably signals through Gαq/11, Gαo, and Gαi, and also Gβγ. PAFR subtypes have not been identified. PAFR$^{-/-}$ mice apparently grow normally. Their phenotypes revealed that the cloned PAFR plays major roles in inflammatory responses including systemic anaphylaxis, but its roles in other biological functions should be clarified by further studies.

PAF, its synthesis, degradation, and cell-surface expression

Platelet-activating factor (PAF), initially recognized as platelet-stimulating activity from FεRI-engaged basophils (*1*), was structurally identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholin in the late 1970s (*2, 3*) (Fig. 1). In contrast to unsaturated fatty acid–derived major autacoid species [*e.g.*, prostanoids (PGs) and leukotriens (LTs)], PAF is unusual in its intact glycerophospholipid structure. The ether-bonded fatty alcohol with C16-18 chain length at the *sn*-1 position of the glycerol backbone, an acetyl residue at *sn*-2, and phosphocholine at *sn*-3 are all required for optimal PAF activity (reviewed in Ref. *4*).

Biological activity of PAF does not seem to be confined to pro-inflammatory functions. Recent works suggest its involvement in a variety of settings, including reproduction, central nervous system functions, and circulatory system disturbance such as atherosclerosis (reviewed in Refs. *5–7*).

The majority of PAF is synthesized from glycerophosphocholins (GPCs) with 1-*O*-alkyl moieties (Fig. 2). 1-*O*-Alkyl-GPCs are enriched with arachidonic acid at the *sn*-2 position (*4*). Upon cell activation, cytoplasmic phospholipase A_2 ($cPLA_2$) (*8*) simultaneously liberates arachidonic acid and Lyso-PAF, the direct precursor of PAF, providing the basis for interrelated synthesis of eicosanoids and PAF. PAF is finally synthesized by the action of acetyl CoA-lysoPAF acetyl transferase. This enzyme has not been purified, and its nature remains to be determined. The involvement of a $cPLA_2$-dependent "remodeling" pathway in bulk PAF synthesis in inflammatory cells was confirmed in $cPLA_2^{-/-}$ mice (*9, 10*). Another metabolic pathway dependent on phosphocholine transfer from CDP-choline to 1-*O*-alkyl-2-acetyl-glycerol was also reported ("*de novo*" pathway, reviewed in Ref. *4*), but its significance remains to be clarified.

PAF is hydrolyzed at the *sn*-2 position by PAF acetyl hydrolases (PAF-AH) to yield lyso-PAF. There exist at least three types of PAF-AH: two intracellular enzymes (tissue types I and II) and one secreted one (plasma type). Tissue type I is a heterotrimer containing the product of the LIS1 gene, which is genetically associated with a congenital brain agyria, Miller-Dieker lissencephaly (*11*). Tissue type II and plasma type PAF-AH are structurally related monomeric enzymes (*12*). Both possess activities hydrolyzing oxidized fatty acyl residues and acetyl residues from the *sn*-2 position of GPCs, and LCAT-like acetyl transferase activity (*13, 14*).

Besides PAF synthesized *via* the regulated pathway, oxidized 1-*O*-acyl GPCs, whose unsaturated fatty acyl resi-

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Abbreviations: PAF, platelet-activating factor; PAFR, PAF receptor; GPCR, G protein–coupled receptor; PG, prostaglandins; LT, leukotrienes; GPC, glycerophosphocholin; cPLA2, cytosolic phospholipase A2; PAF-AH, PAF acetyl hydrolase; TM, transmembranous region; Tg, transgenic.

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dues at the *sn*-2 position are randomly fragmented by oxidization, also stimulate PAF receptor (PAFR) (*15, 16*). The oxidized GPCs possess hydroperoxy fatty acids of shorten chain length (C2–C4), resembling the short acetyl moiety at the *sn*-2 position of PAF (*17, 18*). The oxidized GPC species are implicated in atherogenesis: GPCs with short oxidized fatty acyl moieties are found in oxidized low-density lipoprotein (LDL) (*18, 19*), PAFR is expressed on atherosclerotic lesions in humans (*20*), and intervention of the PAF-like action with PAF-AH or with PAF antagonists successfully suppressed progression of atherosclerosis in model animals (*21, 22*).

Lipid autacoid release across the plasma membrane sometimes requires specific machinery as seen in LTC_4 transport *via* ATP-binding cassette transporter (*23*). In the case of PAF synthesized in vascular endothelial cells, its polar head translocates to the outer surface of the cell *via* undefined "flip-flop" mechanisms, with the saturated alkyl moiety being inserted into outer leaflet of plasma membrane. The cell-associated PAF functions as a juxtacrine liand stimulating adherent leukocytes (reviewed in Refs. *6* and *24*). These characteristics of PAF are reminiscent of fractalkine, a transmembranous chemokine expressed on the endothelial surface (*25*), which induces firm adhesion and trans-endothelial migration of leukocytes through "inside-out" integrin activation. Such short-range PAF signaling may represent mechanisms to avoid its accelerated conversion to inactive lyso-PAF by high activity of plasma-type PAF acetyl hydrolase (*26*).

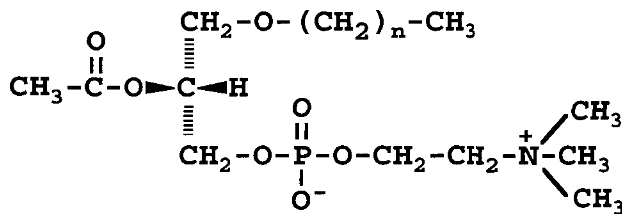

Fig. 1. **Structure of PAF.**

PAF receptor: structure–function analysis and regulated expression

As suggested by earlier findings that PAF specifically binds to and stimulates GTPase activity in polymorphonuclear leucocyte (PMN) membranes (*27*), cloned PAF receptors from various species possess a typical structure of G protein–coupled receptors (GPCRs) with seven transmembrane helices (TMs) (*28–30*) (Fig. 3). To date no other subtypes have been recognized. Specific binding of PAF or PAF antagonists has been detected in various cells including PMNs, platelets, macrophage-lineage cells (Mø, Kupffer cells and microglia), thoracheal epithelium, vascular endothelium, and myometrium (see references in Ref. *5*). PAFR expression in primary T and B lymphocytes is still controversial. PAFR mRNA is widely distributed in PMNs, spleen, kidney, liver, heart, skeletal muscle, and brain from various species. *In situ* hybridization detected PAFR mRNA in mesangial cells in rat kidney, blood vessels, smooth muscles, and alveolar wall in human lung, microglia and to a lesser extent in neurons in rat brain (*5*).

PAFR mutagenesis studies have provided several insights into G protein–coupling, ligand-binding, and activation states of the receptor (Fig. 3). Overexpression of PAFR 3rd intracellular loop, a putative Gq/11 coupling site in m3 muscarinic Ach receptor (*31, 32*), exerts dominant negative effects on PAFR functions (*33*). Mutagenesis of the amphipathic α helix at the 3rd loop [residues 210–220, IHTLLTRPVRQ (rat PAFR); see Fig. 3] disrupted the PAFR-phospholipase C cascade, thereby indicating that the 3rd loop is involved in G protein–coupling (*34*). In addition, A230E exchange at the C-terminal end of the 3rd loop interrupts

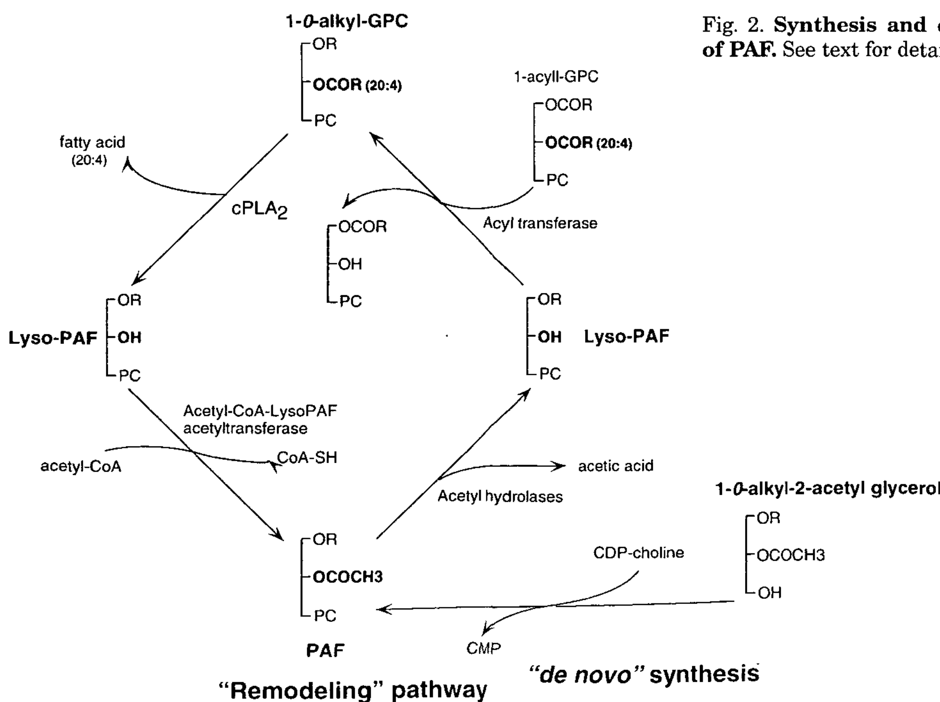

Fig. 2. **Synthesis and degradation of PAF.** See text for detail.

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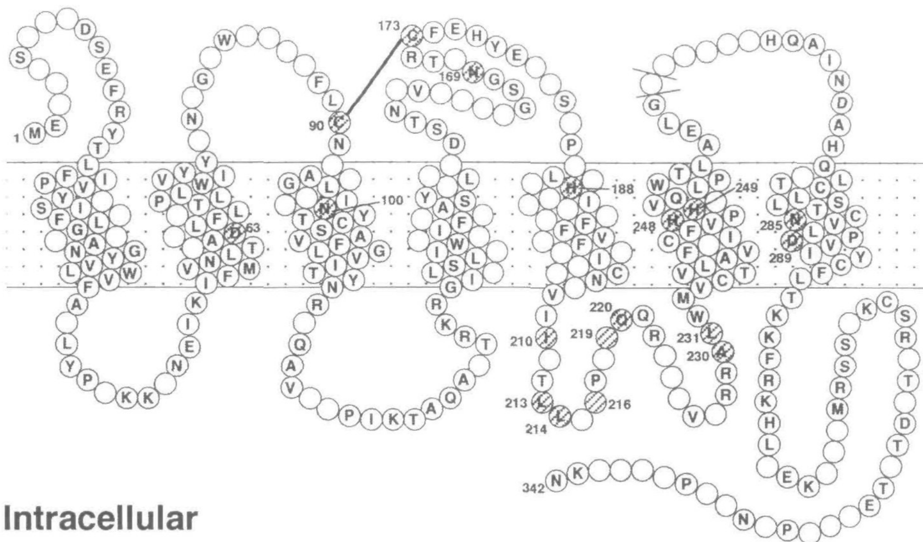

Fig. 3. **Transmembrane structure of PAF receptor.** Shaded amino acids indicate the sites of mutagenesis studies. See text for detail.

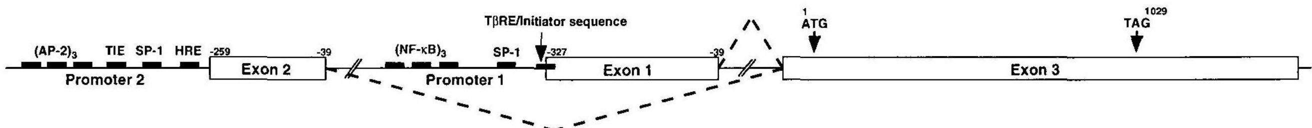

Fig. 4. **Genome structure of human PAF receptor.** Two 5′ non-coding exons (exons 2 and 1; the order is inverted for historical reasons) are spliced to exon 3, the entire coding sequence, yielding two PAFR transcripts. Their expression is regulated by two promoters (promoters 2 and 1). Promoter 2 contains AP-2, TIE (TGFβ-inhibitory element), SP-1 and HRE (hormone responsive element); and promoter 1, NF-κB SP-1, and TβRE (TGFβ responsive element).

PAFR-G protein–coupling (*35*). Interestingly, the adjacent L231R substitution created constitutively active PAFR with intact PAF responsiveness and higher affinity to PAF than wild-type PAFR (*35*). These data suggest that subtle structural changes at around the 3rd loop partially imitate an activation state of PAFR. N100A substitution in the 3rd TM was found to induce another constitutive PAFR activation with higher affinity to PAF (*36*). This seemingly remote effect suggests that G protein activation is defined in three dimensions as well.

Ishii et al. performed Ala-scanning mutagenesis of transmembranous polar amino acids (*36*). They showed that extinction of the polarities in the 2nd, 3rd, and 7th TMs induces higher PAF binding affinities than WT PAFR, whereas replacement of three His residues close to the outer surface in the 5th and 7th TMs critically decreased affinity to PAF (*36*). They proposed that the three His residues coordinately bind phosphate of PAF. These findings are consistent with the idea that ligand-binding pockets in GPCRs are composed in three dimensions of multiple TMs through polar and non-polar interactions. In the GPCR superfamily, D63 in the 2nd TM and N285 and D289 are well preserved (*37*) and hypothetically create a negatively charged binding pocket. This module was once presumed to create a choline-binding pocket (*38*). However, mutagenesis studies showed that these amino acids are not essential for PAF binding (*39*). The binding site for the choline residue of PAF is still undetermined.

PAFR is post-translationally modified by disulfide bonding at C90-C173 and by *N*-linked glycosylation at N169. These modifications are required for efficient cell surface expression of PAFR (*40*). The Ser and Thr cluster at the C-terminus is phoshorylated upon PAF binding, and this process, presumably catalyzed by G protein–coupled receptor kinase (GRK)–2, seems crucial for homologous desensitization and for facilitated internalization of PAFR (*41–44*). Common and downstream desensitization mechanisms are also noted in the PAFR system, including phospholipase Cβ3 (PLCβ3) phosphorylation by protein kinase C (PKC) (*45*) and Gq-mediated proteolysis of inositol 1,4,5-trisphosphate (IP3) receptor (*46*).

PAFR expression seems to be differentially regulated by two promoters (promoters 1 and 2) flanking two 5′-noncoding exons (exons 1 and 2) (*47*) (Fig. 4). These noncoding exons are spliced to an acceptor site on the exon 3 encoding entire PAFR open reading frame, yielding two PAFR transcripts (transcripts 1 and 2). PAFR transcript 1 is ubiquitously expressed and abundant in PMNs and monocytes. Transcript 2 is seen in organs including heart, lung, spleen, and kidney, but its expression is low in PMNs and monocytes (see references in Refs. *5* and *48*). Promoter 1 contains consensus sequences for NF-κB and Sp1 and a TGF-β responsive element, and PAFR expression is augmented in response to phorbol ester and TGF-β (*47, 49*). Promotor 2

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contains a TGF-β inhibitory element and a hormone-responsive element, and transcript 2 levels are regulated negatively by TGF-β and positively by steroid hormones such as retinoic acid, triiodothyronine and estradiol (*50, 51*).

Signal transduction from PAF receptor

Selective PAFR coupling with heterotrimeric G proteins has been studied through various approaches. PAFR regulates initial GPCR 2nd messengers: it augments inositol 1,4,5-trisphosphate (IP_3) synthesis and calcium mobilization and suppresses forskolin-stimulated cAMP synthesis in CHO cells (*52*). The latter effect, a hallmark of Gαi species, is completely inhibited by pertussis toxin (PTX) (*52*). IP_3 synthesis is partially sensitive to PTX in CHO cells and in RBL mast cells (*42, 52*), and the PTX-insensitive portion is abolished when GDP-βS is incorporated into RBL cells, indicating that both PTX-insensitive and sensitive G proteins regulated this pathway. Recently PTX-insensitive Gαq was found to reconstitute the PAFR-IP_3 axis in COS cells (*53*), showing the roles of the Gαq/11 family.

Additional information was obtained from studies focusing on PAFR-induced Erk and p38 MAP kinase activation. PAFR-mediated Erk activation, and also Erk-dependent cytosolic phospholipase A2 activation, are largely sensitive to PTX in CHO cells (*52*). The Erk pathway is dependent on Gαo expression, and PAFR induces azido-GTP incorporation into Goα in CHO cells (*54*). Moreover, expression of a PTX-insensitive mutant of Gαo, but not of Gαi2 or 3, renders the pathway resistant to PTX (*54*). PAFR-induced p38 MAPK activation is insensitive to PTX in CHO cells and in PMNs (*54, 55*). This pathway is attenuated by RGS16 Gα GAP expression, and a QL mutant of Gα11 lacking GTPase activity overcomes the inhibitory effects in CHO cells (*54*). Therefore, it is conceivable that PAFR links to Gαq/11, Gαo, and Gαi G proteins. PLCβ activation is presumably transduced mainly by Gαq/11 and partly by Gαo, p38 by Gαq/11, and Erk by Gαq/11, Gαo, and also by Gβγ depending on cell types (see below).

Molecular mechanisms of the post-G protein signaling network that participate in cellular functions, *i.e.*, cell polarization, adhesion and motility, gene expression, and trophic effects, have been the focus of intensive research (reviewed in Ref. *56*) and are beyond the scope of this review. Noticeable characteristics of the network are that the post-G protein signaling is highly dependent on cell-context. For instance, the PAFR-Erk pathway, which probably regulates cell growth and gene expression including inflammatory cytokines, is Ras-independent and PKC-dependent in fibroblasts (*52, 54*), whereas PAFR activates the Ras-Erk pathway in PMNs, presumably through the Gq-Ras GRF pathway (*56*). In addition, PAFR utilizes transactivation of EGF receptor in Erk activation, which is theoretically transduced by Gβγ and forms part of the Ras pathway, in epidermal cells (*57*). The last example indicates PAFR-transactivation of receptor protein tyrosine kinases (PTKs) or non-receptor PTKs including Src family kinases (*58*), but the underlying mechanisms are still elusive. PAFR activates MEK1/2-Erk and MEK3 (and presumably MEK6)-p38 MAP kinases in various cells (*55, 59*), whereas c-Jun N-terminal kinase activation by PAF has been noted solely in primary hippocampal neurons (*60*). PAFR-mediated PIP3 synthesis, which presumably regulates cell polarization/motility and cell survival and growth, utilizes Gβγ-activatable PI3 kinase γ in a macrophage cell line (*61*), while PAFR signals *via* p85/p110 PI3Ks in an erythroleukemia cell line (*62*). PAFR is also reported to regulate other downstream signaling molecules, including PLD, PLCγ, and other small G proteins, Ral and Rap (*63, 64*).

Roles of PAF receptor in pathophysiological conditions: insights from PAF receptor–overexpressing, and PAF receptor$^{-/-}$ mice

Through a number of experiments in animal models, and in several cases in humans, PAF has been implicated in pathophysiological conditions including allergic asthma, endotoxin shock, acute pancreatitis and dermal inflammations such as psoriasis and pruritis (reviewed in Ref. *5*). Recent works suggests the roles of PAFR in atherogenesis (see above). These proposals are based on PAF-induced pathological responses, prevention of the pathological conditions by PAFR antagonists or by PAF acetylhydrolases, and measurement of PAF or PAF-related compounds in pathological regions. To date, however, PAF antagonists have not been applied clinically. Although PAF is conceivably involved in these conditions, it might play modifying roles in them.

Several reports suggest roles of PAF in implantation of embryos. Pre-implantation embryos synthesize PAF, and notably (*65*), pretreatment of embryos with PAF reportedly increases implantation rate in *in vitro* fertilization in humans (*65, 66*). PAF fulfils the requirements for retrograde messengers in neural synapses in that it is a small and diffusible molecule produced in CNS (*67*). Bazan and colleagues have proposed that hippocanpal LTP, and also memory function in animals, involves PAF-regulated events, based on the observations that a PAF antagonist inhibits LTP in the CA1 region and that *in vivo* infusion of an unhydrolizable PAF analog (methylcarbamoyl PAF) into dorsal hippocampus, amygdala, or entorhinal cortex improved memory functions in male Wistar rats (*68, 69*).

Creation of PAFR-transgenic (Tg) mice and PAFR$^{-/-}$ mice have provided insights into several, if not all, of the above-mentioned possibilities (*70, 71*). Since the PAFR-Tg construct used in the studies is driven by β-actin promoter, it should be kept in mind that PAFR transgene expression is different from that in intrinsic PAFR (*70*). PAFR-Tg spontaneously develops melanocyte tumors (*70*), suggesting an direct or indirect melanocyte proliferating potential of PAFR. PAFR$^{-/-}$ mice grow apparently normally. PAFR-Tg progeny are reproducibly smaller than the wild type when either male or female PAFR-Tg heterozygotes are mated with wild-type mice. However, PAFR$^{-/-}$ mice exhibited normal reproductive potential (*71*). Thus PAFR is not essential for reproduction, but an augmented (or ectopic) PAF signal both in embryos and in maternal systems appears to be disadvantageous for fertilization in mice (*70, 71*).

In PAFR$^{-/-}$ mice, intravenous PAF injection does not cause hypotension, and PAF challenge fails to induce calcium mobilization in PAFR$^{-/-}$ PMNs. Hence, these PAF functions are entirely ascribed to the cloned PAFR. PAFR-Tg and PAFR$^{-/-}$ mice display altered behaviors in response to immunological or inflammatory challenges. PAFR$^{-/-}$ mice are extremely resistant to antigen-induced systemic anaphylaxis, including bradycardia, circulatory shock, and lung edema (*71*). PAFR-Tg mice respond more severely to lipopolysaccharide (LPS)-induced endotoxin shock, while

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PAFR$^{-/-}$ mice respond similarly to wild-type mice (*71*). These findings show that PAF plays major roles in type I (and/or III) allergic anaphylaxis and that it enhances the severity of endotoxin shock. PAFR-Tg mice show bronchial hyper-responsiveness to methacholine as well as PAF (*70*). PAFR-Tg mice are significantly sensitive to PAF injection in terms of bronchial constriction, and these effects seem to be indirectly mediated thromboxane A2 and leukotriene D4 (*70*). PAFR$^{-/-}$ mice are also more resistant to hydrochloric acid aspiration–induced lung edema (a model of aspiration pneumonia) than wild type mice (*72*).

Apparently contradictory to previous pharmacological studies (*69*), PAFR$^{-/-}$ mice exhibited normal LTP and showed no obvious abnormality in excitatory synaptic transmission in the hippocampal CA1 region (*73*). These discrepancies might suggest the existence of PAF receptors other than the cloned one, or that PAF antagonists and/or methylcarbamoyl PAF exert effects *via* a different pathway than PAFR, including PAF acetylhydrolase inhibition.

Conclusion

As the first lipid autacoid receptor to be cloned, the cloned PAFR has furnished information on the inflammatory and non-inflammatory actions of PAF and the signaling mechanisms of GPCRs. The accumulated information suggests that PAFR mediates fine modifications of a variety of biological functions in co-operation with other GPCRs such as chemokine and eicosanoid receptors.

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