Platelet-Activating Factor Enhances Urokinase-type Plasminogen Activator Gene Expression in Corneal Epithelium

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Purpose. To determine whether platelet-activating factor (PAF), a lipid mediator that is accumulated in the cornea after alkali burn, induces the gene expression of urokinase-type plasminogen activator (uPA) in the corneal epithelium. Possible signaling mechanisms of uPA gene induction by PAF also were examined.

Methods. Rabbit corneas were cultured with or without PAF. One hour before stimulation, PAF antagonists or other modulators were added to PAF. In some experiments, the corneas were permeabilized to introduce guanosine triphosphate analogs into the corneal epithelial cells. Corneal epithelia were then harvested for Northern blot analysis, nuclear runoff transcription assay, and zymography.

Results. Platelet-activating factor induced uPA mRNA expression in the corneal epithelium. New protein synthesis was not required for the induction of uPA mRNA. The induction was at the level of transcription as shown by nuclear runoff assays. Additionally, both actinomycin D and α-amanitin inhibited the increase in uPA mRNA by PAF. The message was translated into protein, which was secreted into the conditioned medium. An antagonist with high affinity for intracellular PAF binding sites (BN 50730) inhibited uPA gene expression and cellular secretion of the protein. The effect of PAF was not mediated by G proteins and was independent of protein kinase C- and cyclic adenosine monophosphate-dependent signal transduction pathways. Okadaic acid increased the expression of uPA and, at longer times, augmented the effect of PAF, suggesting that a signaling pathway that requires phosphorylation is involved in activated uPA mRNA synthesis.

Conclusions. After corneal injury and inflammation, PAF may be an important initiator of the proteolytic cascade, leading to epithelial defects and corneal ulceration. Antagonists of PAF could be useful in the prevention of these diseases. Invest Ophthalmol Vis Sci. 1996; 37:2037–2046.
remodeling and cell migration. Additionally, the growth factor domain of uPA directs its binding to a membrane receptor, which may be important for the localized pericellular proteolysis.3

Plasmin, the product of PA, is also a serine protease, but it has a very broad spectrum of substrates.4 Although the classic plasmin substrate is fibrin, there are other components of the ECM that can be cleaved by plasmin, e.g., laminin and fibronectin. Plasmin also activates the metalloproteinases (e.g., collagenases).5 Thus, the PA–plasmin system plays an important role in the breakdown of ECM.

Plasminogen activators initiate a proteolytic cascade, making them key participants in a number of physiological and pathologic conditions, such as embryonic development, ovulation, inflammation, wound healing, angiogenesis, and neoplasia.4,6 In the gene expression in the cornea and how these events are regulated.

ECM degradation. Here, we demonstrate that PAF increases uPA gene transcription, followed by cellular release of the uPA protein. We further explore the signaling mechanisms for the activation of the uPA gene by PAF.

MATERIALS AND METHODS

Chemicals

cPAF (1-alkyl-2-n-methylcarbamyl-sn-glycerol-3-phosphorylcholine; Calbiochem, San Diego, CA), prepared in a 1 mM stock solution in ethanol, was divided into aliquots and stored at -20°C. Before use, the solution was diluted 1:100 (vol/vol) in Dulbecco’s phosphate-buffered saline. The PAF antagonists BN 50730, BN 52021, and BN 50727 (kindly provided by Dr. P. Braquet, Institut Henri Beaufour, Le Plessis Robinson, France) were dissolved in dimethylsulfoxide.

Platelet-activating factor (PAF) is one of the most potent lipid mediators. It accumulates in the cornea 30 minutes after alkali injury, increasing rapidly between 1 hour and 3 hours, and it continues to increase up to 24 hours.14 Because infiltrating polymorphonuclear leukocytes appear relatively slowly in the avascular cornea, they would not be a source of PAF until later times after injury. A major complication of corneal alkali injury is ulcer formation, which involves the action of proteolytic cascades comprising collagenases, gelatinases, and PAs. In the corneal epithelium, PAF selectively increases the expression of two metalloproteinases, MMP-1 and MMP-9,15,16 suggesting that PAF may initiate the proteolytic cascade leading to ECM degradation. Here, we demonstrate that PAF induces uPA gene transcription, followed by cellular release of the uPA protein. We further explore the signaling mechanisms for the activation of the uPA gene by PAF.

Corneal Organ Culture

All experimental procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rabbit eyes (Pel- Freeze, Rogers, AR) were maintained on ice until delivery to the laboratory. The corneas were dissected and cultured in serum-free Eagle’s minimum essential medium as described.15 Corneas (two per flask) were incubated for 1 hour before 100 nM cPAF was added, and incubations continued for the times specified in each experiment. In the experiments with PAF antagonists, the corneas were incubated for 1 hour in the presence of 10 µM of the antagonists before cPAF was added to the culture medium. The final concentration of vehicles (dimethylsulfoxide and ethanol) added to the culture media was less than 0.01% and did not affect the expression of the genes studied in the cornea. Other agents were added as described in each experiment.

In the experiments with G protein inhibitors, the concentrations of pertussis toxin added were 200 and
300 mg/ml, and incubations were conducted for 2, 4, and 24 hours before the addition of cPAF. In the experiments with cholera toxin (10 μM) or isotretadrine (15 μg/ml), the inhibitors were added 4 hours before PAF stimulation. To allow intracellular access of guanine nucleotide analogs, corneal epithelial cells were permeabilized by transient hypo-osmotic treatment.\(^{17,18}\) Intact corneas were incubated in hypotonic buffer (3 mM Hepes, 3 mM Mg-ATP, pH 7.4) at 4°C for 5 minutes. The buffer also contained GTP γ-S or GDP-β-S when indicated. In some experiments, corneas were permeabilized with saponin (75 μg/ml). After permeabilization, the experimental procedures were carried out as above. To verify that the guanyl-nucleotides were incorporated into the epithelial cells, \(^{32}\)S-labeled guanine nucleotide analogs were added in a few samples. After incubation, the epithelial cells were scraped and treated with Solvable (NEN). Scintillation counting showed that permeabilized corneal epithelial cells contained 2 to 4 times more radioactivity than nonpermeabilized cells, in agreement with observations in other cells.\(^{17,18}\)

**RNA Extraction and Electrophoresis**

Corneal epithelial cells (two corneas per sample) were scraped with a sterile blade and rapidly homogenized on ice in 0.25 ml of 4 M guanidine isothiocyanate, 25 mM sodium citrate, 0.5% n-lauryl sarcosine, and 0.1% β-mercaptoethanol. RNA was then extracted with phenol–chloroform–guanidine isothiocyanate.\(^{19}\) The yield of RNA per sample was between 50 and 70 μg.

For electrophoresis, RNA samples were diluted to a concentration of 1 μg/μl, and 7 μg from each sample were mixed with 19 μl formamide:formaldehyde:10 X MOPS (5:1.6:1, vol:vol:vol) and denatured for 5 minutes at 65°C. The RNA was separated in a 1.2% agarose gel containing 7.4% formaldehyde. RNA was transferred to nylon membranes by capillary blotting and immobilized by exposure to ultraviolet light for 5 minutes.

**Northern Hybridization**

The hybridization buffer contained 50% formamide, 5 X SSPE (1 X SSPE = 0.18 M NaCl, 10 mM sodium phosphate, 1 mM EDTA; pH 7.4), 5 X Denhardt’s solution, and 150 μg/ml salmon sperm DNA. pHUK8 (uPA cDNA-containing plasmid) was digested with restriction enzyme PstI to give a fragment of 1.5 kb; pHcGAP (GAPDH cDNA-containing plasmid) was digested with PstI and XbaI to give a 0.75 kb fragment. The cDNA fragments were labeled with \(\alpha^{32}\)P-dCTP by random primer extension. GAPDH was used to determine the relative amount of RNA in each lane by rehybridizing the blots that had been probed with uPA. For hybridizations, final specific activity of the probes was 2 X 10⁶ cpm/ml.

After hybridization at 42°C for 18 hours, the membranes were washed in 2X SSPE for 15 minutes at room temperature, 30 minutes at 55°C, and 0.1 X SSPE for 10 minutes at 55°C. For autoradiography, the filters were exposed in a phosphorimager cassette, and the intensity of the bands was quantified (Bio-Rad, Richmond, CA). Values are expressed as the uPA message relative to the amount of GAPDH in each sample.

**Nuclear Runoff Transcription**

The following procedures were performed at 4°C unless otherwise stated. Corneal epithelial cells (30 corneas per sample) were dissected and homogenized in 2 M sucrose, 1 mM MgCl₂, in a glass–Teflon (Dupont, Wilmington, DE) dounce homogenizer (0.075 mm clearance). After filtration through a 100 μm Teflon mesh, the homogenate was centrifuged at 64,000 g for 30 minutes. The pellet was resuspended in the same homogenizing buffer and overlaid with 1.8 M sucrose–1 mM MgCl₂ solution. The sample was centrifuged at 85,000 g for 30 minutes. The pellet was washed twice with 50 mM Tris HCl, 5 mM MgCl₂, 0.1 mM EDTA, pH 8.4 (storage buffer), and the nuclei were resuspended in the same buffer containing 40% glycerol, rapidly frozen in liquid N₂, and kept at −80°C until use.

Nuclear runoff assays were performed in a final volume of 200 μl with 100 μl of reaction buffer (10 mM Tris HCl, pH 8, 5 mM MgCl₂, 300 mM KCl, and 1 mM each of ATP, CTP, and GTP) containing 6 X 10⁶ nuclei and 0.5 μCi \(\alpha^{32}\)P UTP (800 Ci/mmol; NEN–Dupont) per sample. The reaction was followed for 30 minutes at 30°C, stopped by adding DNase I in HSB buffer (0.5 M NaCl, 50 mM MgCl₂, 2 mM CaCl₂, 10 mM Tris HCl, pH 7.4), and continued to incubate for 5 minutes. RNA was isolated using buffered phenol:chloroform:isoamyl alcohol (25:24:1). uPA and GAPDH cDNA fragments (5 μg each) were deposited on nylon membranes, which were cut into strips and prehybridized in 0.5 M NaH₂PO₄, 1% SDS (sodium dodecyl sulfate), 0.1 mM EDTA, pH 7.2, at 42°C for 30 minutes. For hybridization, equal amounts of labeled RNA (4 X 10⁶ cpm) from each nuclear preparation were added to 1 ml hybridization buffer (10 mM Tris, 2% SDS, 10 mM EDTA, 600 mM NaCl, pH 7.4) The incubation was carried out at 42°C for 48 hours. After hybridization, the strips were washed in 2 X SSPE 0.1% SDS at 65°C for 2 hours, then in 0.1 X SSPE and 0.1% SDS at 55°C for 20 minutes. Hybridization signals were detected and quantitated by a phosphorimager as described above.

**Zymography**

New Zealand albino rabbits weighing 2 to 3 kg were anesthetized and killed with an overdose of pentobarbital. The corneas were dissected and cultured for 12 or 24 hours in the presence of cPAF with or without the PAF antagonist as described above.
Aliquots of the culture medium (12.5 μl) were diluted with Laemmli buffer (1:1), and zymography was performed using a modified method of Roche et al. SDS gels (8%) were prepared using a mixture of acrylamide:biacrylamide, 29:1 (Bio-Rad) and contained 0.06 μg/ml plasminogen and 0.1% casein.

Electrophoresis was performed at 4°C using a Bio-Rad mini-slab gel box with a constant voltage of 80 V. Then the gels were washed in a 2.5% solution of Triton X-100 for 1 hour at room temperature to remove SDS and were developed by incubation in reaction buffer (50 mM Tris, pH 7.5, 10 mM NaCl) at 37°C for 6 hours. The gels were then stained with Coomassie brilliant blue (Sigma), which revealed the position of the uPA that activated plasminogen to degrade casein as clear bands. For quantitation, the gels were photographed using Kodak film (Technical Pan 4415; Eastman Kodak, Rochester, NY). The negatives were captured by Eagle Eye Still Video System (Stratagene Cloning Systems, La Jolla, CA) and quantitated by QGEL Electrophoresis Gel Imaging and Analysis System (QuantiGel, Madison, WI).

Data Analysis
Experiments were performed in triplicate and were repeated at least twice. In most cases, results are expressed as mean ± SD. Data were analyzed by two-tailed Student’s t-test, and significance was determined at P < 0.05.

RESULTS
Platelet Activating Factor-Induced Expression of uPA and Effects of Platelet Activating Factor Antagonists
Nonstimulated epithelial cells from organ-cultured corneas expressed low levels of uPA mRNA (Fig. 1). This expression increased as early as 2 hours after cPAF stimulation, remained elevated up to 8 hours, and returned to control level by 24 hours (Fig. 1). Although the rabbit uPA gene has not yet been cloned, the size of uPA mRNA has been determined to be approximately 2.2 kb, which coincides with the size of uPA mRNA from other species.11

Corneas preincubated for 1 hour with 10 μM PAF antagonist BN 50730 displayed complete inhibition of PAF-induced uPA expression at all times of incubation (Fig. 2). Other antagonists with affinities for diverse PAF-binding sites21 also were tested. BN 52021 and CV3988 did not significantly inhibit the expression of uPA mRNA. However, BN 50727 and WEB-2086 effectively blocked uPA expression (Fig. 2). These different actions could be attributed to the different potencies of the antagonists for intracellular binding sites, with BN 50730 the most specific and efficient for intracellular (microsomal) binding sites.22 The antagonists alone did not have any effect on uPA mRNA expression.

Platelet Activating Factor-Induced uPA Expression Is Independent of G Proteins
The cell membrane PAF receptor is a seven-transmembrane domain protein with regions for interaction with G proteins.21 Pertussis toxin, which inhibits G protein signaling pathways by adenosine diphosphate-ribosylating Gα protein, failed to block the accumulation of uPA mRNA by cPAF during incubations of
Platelet Activating Factor Promotes the Accumulation of uPA mRNA by Activating Transcription Without Requiring New Protein Synthesis

Platelet activating factor is a transcriptional activator of c-fos and c-jun, and this effect does not require new protein synthesis. The rapid induction of uPA mRNA (2 hours) by PAF suggests an event similar to that elicited by the lipid mediator on primary response genes.

The induction of uPA mRNA by cPAF was not inhibited in corneas pretreated for 1 hour with cycloheximide (Fig. 3). This indicates that the induction of the gene by PAF is independent of new protein synthesis. Moreover, there is an increase in the expression of uPA after cycloheximide treatment, suggesting that certain labile protein factors may be required for the repression of uPA gene transcription or the degradation of uPA mRNA.

In other tissues, TPA induces uPA synthesis by transcriptional activation. In corneal epithelial cells, up to 24 hours. Similar results were obtained using another G protein inhibitor, isotetrandrin. Cholera toxin, originally identified as an activator of G, was found in recent years to have inhibitory interactions with G proteins. Notably, it was shown to inhibit PAF receptor-coupled G proteins in certain cultured cells. In corneal epithelium, however, cholera toxin did not affect the induction of uPA mRNA by PAF. In permeabilized corneal epithelial cells (see Materials and Methods), the G protein stimulator GTPγS did not increase the expression of uPA even at concentrations as high as 300 µM, although it was effectively introduced into the cells. In addition, the G protein inhibitor, GDP-β-S, at 400 and 500 µM, did not inhibit the response induced by cPAF in permeabilized cells (data not shown).

FIGURE 2. Effect of platelet activating factor (PAF) antagonists on PAF-induced urokinase-type plasminogen activator (uPA) mRNA expression. (A) Northern blot hybridization showing that BN 50730 (1 hour before incubation, 10 µM) blocks the effect of PAF on uPA induction after 2 and 8 hours of incubation. (B) Histogram showing the effects of different PAF antagonists on PAF-induced uPA mRNA expression. Corneas were preincubated for 1 hour with 10 µM of each of the PAF antagonists and then stimulated with 100 nM cPAF for 2 hours. Northern blots were quantitated by the phosphoimager. Values of uPA mRNA intensity from each sample were normalized to that of GAPDH. The values shown in the graph are mean ± SD of three samples. The experiment was repeated twice with similar results. *Statistically significant increase over control. **Statistically significant inhibition compared with cPAF-treated samples. NS = no significant differences.

FIGURE 3. Cycloheximide potentiates urokinase-type plasminogen activator (uPA) mRNA expression by platelet activating factor (PAF) and tissue-type plasminogen activator (TPA). Corneas were pretreated with 30 µM cycloheximide for 1 hour before adding cPAF (100 nM) or TPA (200 nM), and incubation continued for 2 or 8 hours. Total RNA was then prepared and analyzed by Northern blot. Autoradiographs were quantified by phosphoimaging. The values shown in the graph represent band intensity of uPA mRNA relative to that of GAPDH and are the mean ± SD of three samples. The experiment was repeated twice.
200 nM TPA effectively induced uPA (Fig. 3). Cycloheximide also enhanced uPA mRNA accumulation by TPA. These experiments demonstrate that the mechanisms of PAF and TPA induction of uPA mRNA in the cornea share similarities. However, they follow different time courses of activation: After PAF stimulation, uPA mRNA in the corneal epithelium returned to the control level by 24 hours (see Fig. 1B), whereas in TPA-treated corneal epithelium, the mRNA remained elevated up to 24 hours (data not shown).

To establish whether the effect of PAF on uPA mRNA levels reflects enhanced transcription, several experiments were performed. Nuclear runoff assays showed that there was increased synthesis of uPA mRNA 2 hours after cPAF treatment (Fig. 4). Corneas incubated for 1 hour with 10 μM actinomycin D, an RNA synthesis inhibitor, and then stimulated with cPAF showed complete inhibition of the PAF effect (Fig. 5). Actinomycin D also inhibited the TPA-induced uPA gene expression. Pretreatment of corneas for 6 hours with α-amanitin (50 μg/ml), which binds to RNA polymerase II and blocks mRNA synthesis, also inhibited the effect of PAF on uPA (data not shown). These results indicate that in the corneal epithelium, an important mechanism for PAF to stimulate uPA mRNA expression is to increase transcription of the gene.

Platelet Activating Factor Induction of uPA Is Independent of Cyclic Adenosine Monophosphate and Protein Kinase C Pathways and Is Potentiated by Okadaic Acid

Several studies have shown that the uPA gene regulation is complex, involving different signal transduction pathways. To examine the signaling mechanisms in PAF-induced uPA expression, we studied several activators and inhibitors of protein phosphorylation. The increased expression of uPA by TPA was blocked by 100 nM staurosporine, a protein kinase C inhibitor (data not shown). There was no additive effect on uPA mRNA induction when TPA and PAF were added together. The PAF antagonist BN 50730 did not inhibit the effect of TPA, and staurosporine did not inhibit the effect of PAF on uPA. Dibutryl cAMP and bromo-cAMP, both lipid-soluble cAMP derivatives, did not induce uPA mRNA in corneal epithelium. Similarly, H-89, a protein kinase A inhibitor, did not modify the effect of PAF (not shown). To determine whether protein phosphatases can modulate the expression of the uPA gene in the cornea, 100 nM okadaic acid, a selective inhibitor of protein phosphatases 1 and 2A, was used in the presence or absence of cPAF (Fig. 6). Okadaic acid slightly but significantly increased the induction of uPA mRNA at 2, 4, and 8 hours, suggesting that phosphorylation is involved in uPA gene regulation. Okadaic acid also potentiated the effect of PAF at 8 hours but not at 2 or 4 hours, reflecting the effect of accumulated phosphorylation and indicating that a protein phosphatase is involved in turning off the uPA gene induction.

Induction of uPA Enzymatic Activity by Platelet Activating Factor

The induction of uPA mRNA by PAF was followed by increased activity of uPA released into the medium. In Figure 7A, the clear bands seen against the dark background correspond to an apparent molecular weight of 46 to 48 kDa, in agreement with the molecular weight...
PAF Enhances uPA Gene Expression in Corneal Epithelium

Platelet activating factor exerts many of its effects through a receptor containing seven transmembrane domains that belong to the superfamily of G-protein-coupled receptors. There are also intracellular binding sites for PAF linked to gene activation. We have demonstrated that BN 50730, an antagonist that binds selectively to intracellular PAF binding sites, blocked PAF induction of uPA completely. On the other hand, some PAF antagonists, such as BN 52021, with low affinity for intracellular binding sites do not block PAF-induced uPA expression. Prolonged incubation of uPA species.

Platelet activating factor increased uPA enzymatic activity in the culture medium at 12 and 24 hours. The PAF antagonist BN 50730 blocked the PAF-enhanced uPA enzymatic activity (Fig. 7).

DISCUSSION
We have demonstrated that, in the rabbit corneal epithelial cells, PAF induces transcription of the uPA gene, which is followed by secretion of the uPA protein.
Conditioned medium

MW (kDa)

-52
-37

uPA

BN50730

Hours

12
24

+ + + + +

+ + + + +

+ + + + +

+ + + + +

+ + + + +

+ + + + +

+ + + + +

+ + + + +

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+ + + + +

FIGURE 7. Platelet activating factor (PAF) increases urokinase-type plasminogen activator (uPA) protein in the conditioned medium of organ-cultured cornea. (A) Zymography showed that the conditioned medium of PAF-treated corneas contained more uPA activity than the respective controls, and the effect was blocked by the PAF antagonist BN 50730. Rabbit corneas were cultured in the presence or absence of cPAF and BN 50730. Twelve and 24 hours after incubation, aliquots of the conditioned medium were diluted with Laemelli buffer (1:1). Twenty-five microliters was loaded in each lane and subjected to SDS-PAGE as described in Materials and Methods. After development, a clear band of activity was seen at approximately 46 kDa. (B) Histogram showing that PAF increased uPA protein in the conditioned medium of PAF-treated corneas. Values shown in the graph represent the mean ± SD of three experiments.

The results from nuclear runoff experiments, along with the effects of actinomycin D and α-amanitin, suggest that the increased expression of uPA occurs primarily through enhanced transcription of the gene. Although the transcription factors in the activation of uPA by PAF are unknown, c-Jun may be involved. Unlike c-Fos, c-Jun displays constitutive expression in the corneal epithelium, and it is activated by PAF. Several kinases are known to phosphorylate c-Jun, such as the mitogen-activated protein (MAP) kinase. MAP kinase activity is increased by PAF and blocked by BN 50730 in the corneal epithelium. It is possible that the activated MAP kinase phosphorylates c-Jun, which, in turn, becomes activated and subsequently increases transcription of uPA. The possible links between these events are under investigation.

c-Jun is involved in the expression of uPA mRNA after okadaic acid treatment in the renal epithelium cell line LLC-PK1. Okadaic acid stabilizes c-Jun by inhibiting its dephosphorylation. Okadaic acid also increased uPA mRNA expression in the corneal epithelium, and, at longer times of incubation (8 hours), there was an additive effect with PAF, further suggesting the involvement of c-Jun in the activation of uPA by PAF.

The uPA gene is modulated by a variety of extracellular signals, including tumor necrosis factor, follicle-stimulating hormone, and basic fibroblast growth factor, depending on the cell types. These extracellular stimuli generally converge on two signaling pathways: the protein kinase A (PKA) and protein kinase C (PKC) pathways. The promoters of the uPA gene have a cAMP-responsive element, at nucleotide position −3500 with respect to the initiation site, that mediates PKA activation, as well as a PEA-3/AP-1 site at −2100 that mediates PKC and okadaic acid activation.
The rabbit uPA gene has not yet been cloned. Nevertheless, our results indicate that even though a PKC-dependent pathway is present in the rabbit corneal epithelial cells, the activation of uPA by PAF may not involve such a pathway. Stauroporin, which blocked the activation of uPA by TPA, did not block the effect of PAF. On the other hand, the cAMP–PKA-dependent pathway is perhaps not involved in the activation of uPA in the corneal epithelium, indicated by the fact that neither dibutyryl-cAMP nor bromo-cAMP stimulated the expression of uPA; and H89, a protein kinase A inhibitor, did not inhibit PAF-induced uPA expression. Additionally, okadaic acid and PAF may share a common cis-element of the uPA promoter (e.g., AP-1) for the activation of uPA.

A significant increase in uPA activity was detected in the medium at 12 hours and 24 hours after cPAF treatment. This effect was blocked by BN 50730. These results suggest that uPA protein is secreted after PAF treatment. Because the corneal organ culture has other cell types, the possibility that keratocytes or endothelial cells also contributed to the uPA in the medium cannot be excluded. Although it was our argument that using whole cornea in the experiment has the merit of mimicking more closely in vivo situations than cultured cells do, the possibility of epithelial-stromal interactions during the incubation cannot be discarded. Nevertheless, if such interactions do occur, it is also possible in vivo and will not mask the effect of PAF, a mediator generated locally. In fact, PAF, injected in vivo into the rabbit corneal stroma, induces not only collagenase mRNA expression but also uPA mRNA in the corneal epithelium (Tao, unpublished data, 1995).

In summary, the data presented in this article indicate that PAF could be one important initiator of the extracellular matrix proteolytic degradation. Platelet activating factor enhances uPA gene expression through transcriptional activation in the corneal epithelium from organ-cultured whole cornea. This increase of uPA message is mediated by intracellular PAF binding sites but is not mediated by G protein-coupled receptors. The induction requires protein phosphorylation; however, it is probably independent of protein kinase C or protein kinase A pathways. In light of the effect of PAF on inducing other matrix-degrading enzymes, overactivation of these proteolytic pathways by PAF may lead to corneal epithelial defects and ulcerations. In addition, PAF, with its potent chemotactic properties, may attract polymorphonuclear leukocytes and other leukocytes to the site of injury, initiating subsequent degranulation and contributing to the increase in various proteases and other degrading enzymes. The mechanism of uPA induction by PAF described here also could play a role in other inflammatory–injury conditions in which proteases cause destruction of the extracellular matrix, such as in rheumatoid arthritis and bullous pemphigoid. In these circumstances, PAF antagonists could be of therapeutic value by limiting the breakdown of the extracellular matrix and reducing tissue destruction.

**Key Words**

corneal epithelium, extracellular matrix, platelet-activating factor (PAF), signal transduction, urokinase-type plasminogen activator (uPA)

**References**


