Factor VIIa stimulates endothelin-1 synthesis in TNF-primed endothelial cells by activation of protease-activated receptor 2

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A B S T R A C T

The mechanisms linking prothrombotic changes to endothelial dysfunction and accelerated atheroma formation have yet to be fully defined. Expression of TF (tissue factor) on the endothelium is potentially an initiating event as binding and activation of FVII (factor VII) can result in thrombosis. Although PAR2 (protease-activated receptor-2) is expressed on vascular endothelium, its precise physiological significance and mechanism of activation have yet to be defined. In the present study, we investigated whether PAR2 can be activated by FVIIa (activated FVII) and induce ET-1 (endothelin-1) synthesis. In bovine aortic endothelial cells pretreated with TNF (tumour necrosis factor-α) to increase TF expression, FVIIa stimulated ET-1 synthesis via activation of PAR2. Although FX (factor X) alone was inactive, this response was enhanced by using FVII and FX in combination. Inhibition of the proteolytic activity of FVIIa abolished the response. The PAR2 agonist peptide SLIGKV also enhanced ET-1 release on TNF-pretreated cells. The response to FVIIa was inhibited by a PAR2 antagonist peptide FSLLRY. Inhibition of the p38 MAPK (mitogen-activated protein kinase) reduced PAR2 expression and the ET-1 response. In summary, FVIIa can stimulate ET-1 synthesis in endothelial cells by activating PAR2, demonstrating a potential link between thrombotic processes and endothelial cell dysfunction.

INTRODUCTION

The response-to-injury hypothesis of atherosclerosis proposes that endothelial dysfunction is the first step in atherosclerosis [1]. This endothelial dysfunction leads to the development of a prothrombotic state characterized by the expression of TF (tissue factor), which is a 47 kDa transmembrane glycoprotein [2]. Endothelial cells do not constitutively express TF, but its expression can be induced by cytokines [3]. TF initiates blood coagulation by binding FVII (factor VII). FVIIa (activated FVII) can induce gene expression on binding with TF [4]. This TF/FVIIa complex activates factor IX and FX (factor X), triggering the coagulation cascade which, if unrestrained, ultimately leads to thrombus formation [2]. Prothrombotic states are strongly linked to the classical risk factors for atherogenesis [5,6]; however, the mechanisms linking prothrombotic changes to endothelial dysfunction and atheroma formation have not been clearly described.

Pathological studies have identified TF present in cells within atherosclerotic plaques, including smooth muscle cells, foam cells and monocytes, and in the extracellular matrix [7]. Furthermore, the endothelium overlying atherosclerotic plaques expresses TF [8]. Therefore the atherosclerotic vessel may trigger blood coagulation,

Key words: endothelial dysfunction, endothelin-1 synthesis, factor VIIa, protease-activated receptor (PAR), thrombosis, tissue factor, tumour necrosis factor.

Abbreviations: FVIIa, activated FVII; BAEC, bovine aortic endothelial cells; DMEM, Dulbecco's modified Eagle's medium; ET-1, endothelin-1; FVII, factor VII; FX, factor X; FXa, activated FX; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PAR, protease-activated receptor; rFVIIa, recombinant FVIIa; TF, tissue factor; TNF, tumour necrosis factor-α.

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especially if a plaque ruptures exposing TF to flowing blood. The expression and activity of extracellular TF within atherosclerotic plaques has been localized to shed membrane microparticles derived from monocytes and lymphocytes in close proximity to apoptotic cells [9]. In addition, raised levels of procoagulant endothelial microparticles have been found in the blood of patients with acute coronary syndromes, which suggests an important role for endothelial injury in generating prothrombotic states [10].

There is increasing evidence for the peptide ET-1 (endothelin-1) playing a key role in atherogenesis. Its effects on vascular smooth muscle include vasoconstriction and mitogenic activity, which is important in atheroma formation [11]. ET-1 is abundant in human coronary artery atherosclerotic lesions [12], and patients with symptomatic atherosclerosis show higher plasma levels of ET-1 with a significant correlation with the number of sites of disease involvement [13]. ET-1 is localized to endothelial cells overlying atherosclerotic plaques and is virtually absent from adjacent endothelium [14]. Evidence suggests that ET-1 accounts for most of the resting tone of atherosclerotic coronary arteries [15]. Indeed, ET-1 antagonists can reverse endothelial dysfunction in hypercholesterolaemia and prevent atherosclerotic lesion formation in experimental models [16].

The PAR (protease-activated receptor) family of G-protein-coupled receptors consists of four distinct receptors. Thrombin activates PAR1, PAR3 and PAR4 [17]. Although trypsin and other proteases activate PAR2 [18], the precise stimuli involved in its physiological activation have yet to be identified. These receptors are activated by cleavage of the N-terminus to expose a tethered ligand, which binds and activates the receptor. PAR1 mediates thrombin signalling in platelets and endothelial cells. Agonists of PAR2 have been shown to induce inflammation, NO (nitric oxide)-dependent vasodilatation and release of inflammatory cytokines [19]. PAR2 has also been shown to mediate signalling in response to FVIIa when TF is co-expressed in the same cell [18].

In the present study, we have investigated whether FVII can induce ET-1 synthesis by binding to TF and activating PAR2 on the endothelial cell surface, thereby linking prothrombotic states to vascular dysfunction and atherogenesis.

METHODS

Materials

rFVIIa (recombinant FVIIa) was a gift from Novo Nordisk Pharmaceuticals. Inactivated FVII was prepared by blocking the active site of rFVIIa by addition of a 2-fold molar excess of *H*-D-Phe-Phe-Arg-chloromethylketone (Bachem). FVII, FX and FXa (activated FX) were from Enzyme Research Laboratories. The PAR1 agonist SFLLRN (Ser-Phe-Leu-Leu-Arg-Asn), PAR2 agonist SLIGKV (Ser-Leu-Ile-Gly-Lys-Val) and PAR2 antagonist FSLLRY (Phe-Ser-Leu-Leu-Ary-Tyr) were purchased from Bachem. All other reagents were from Sigma.

Cell culture and experimental conditions

BAEC (bovine aortic endothelial cells) were grown in DMEM (Dulbecco's modified Eagle's medium) with 4 mmol/l glutamine and supplemented with 10 % (v/v) FCS (fetal calf serum), 100 units/ml penicillin, 68 μmol/l streptomycin, 2.7 μmol/l amphotericin B and 25 mmol/l HEPES at 37 °C in a humidified CO_2 incubator (95 % air, 5 % CO_2). Cell culture medium was changed 18–24 h before each experiment. Experiments were performed on confluent cultures with test substances diluted in serum-free medium.

For experiments on ET-1 release using rFVIIa and PAR agonists, cells were pretreated with 10 ng/ml TNF (tumour necrosis factor-α) for 2 h, followed by relevant treatments for 4 h, after which the medium was collected for assay.

Desensitization experiments were performed by pretreatment with TNF and 100 μmol/l SLIGKV in combination for 2 h. During this 2 h pretreatment period, the medium containing both stimulants was changed after every 30 min. The effect of p38 MAPK (mitogen-activated protein kinase) inhibition was tested by treatment with 2 μmol/l SB203580 for 30 min either before or after TNF treatment. The antagonist peptide, FSLLRY, was used at 100 μmol/l.

ET-1 sandwich immunoassay

ET-1 was measured by using a 'double-recognition site' sandwich immunoassay method as described previously [20]. To exclude cytotoxicity of treatments, cell viability was assessed by microscopy and measured using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; 0.4 mg/ml in DMEM; Sigma] incubated for 1 h at 37 °C to determine mitochondrial dehydrogenase activity.

TF activity assay

This was performed using confluent cultures of BAEC in 96-well plates (Falcon). Cells were washed with PBS and then 100 μl of serum-free DMEM alone or containing 10 ng/ml TNF was added. After incubation for up to 6 h, the cells were washed three times with PBS and 50 μl of 0.2 nmol/l FVII was added in HBSS (Hanks balanced salt solution) containing 2.5 mmol/l $CaCl_2$. This was incubated for 10 min at 37 °C, and 50 μl of substrate [0.2 mmol/l; Boc-Leu-Thr-Arg-MCA (4-methyl-coumaryl-7-amide); Peptide Institute] was added and incubated for 30 min at 37 °C to permit hydrolysis by the TF/rFVIIa complex [21]. TF activity was calculated with reference to AMC (7-amino-4-methyl coumarin)

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standards, and was measured using a fluorimeter (excitation, 360 nm; emission, 460 nm; CytoFluoro II, PerSeptive Biosystems).

Immunofluorescence of PAR2 and phosphorylated p38 MAPK

BAEC were grown on glass coverslips for immunofluorescence labelling. The cells were fixed at room temperature in 4 % paraformaldehyde and then permeabilized with Triton X-100. For PAR2 detection, the cells were incubated for 60 min with a goat polyclonal primary antibody (1 in 200 dilution; Santa Cruz Biotechnology) and then for 60 min with a rhodamine-conjugated donkey anti-goat secondary antibody (1 in 10 000 dilution; Jackson ImmunoResearch Laboratories) at room temperature in the dark. For phosphorylated p38 MAPK detection, the cells were incubated overnight with a rabbit polyclonal primary antibody (1 in 200 dilution; Cell Signalling Technologies) and then for 60 min with a donkey anti-rabbit secondary antibody (1 in 100 dilution; Jackson ImmunoResearch Laboratories).

Measurements of preproET-1 and TF mRNAs

BAEC were grown in 24-well plates. Total RNA was extracted using the Strataprep Total RNA Miniprep Kit (Stratagene) and quantified using Ribogreen dye (Molecular Probes). cDNA was prepared using AMV Reverse transcriptase (Promega) and random hexamers [22]. Levels of mRNA were quantified by real-time PCR using the ABI PRISM® 5700 Sequence Detection System (Applied Biosystems) and the *Taq*Man® Universal PCR Master Mix (PerkinElmer Applied Biosystems). The probes and primers were designed using Primer Express software (PerkinElmer Applied Biosystems). Bovine ET-1 forward primer, 5′-AAGAAGTGTGTCTACTTCTGCCATCTG-3′, and reverse primer, 5′-AAAGAAGTCCTTTAAGGAGCGCT-3′; and *Taq*Man probe, FAM-5′-TGGGTCAACACTCCAGAGCACGTTGTT-3′-TAMRA, where FAM and TAMRA are 6-carboxyfluorescein and 6-carboxytetramethylrhodamine respectively. Bovine TF forward primer, 5′-TTTCCTACCCCGCAGACACT-3′, and reverse primer, 5′-CTAGGTAGGGTGTGAACTCCGG-3′; *Taq*Man probe, FAM-5′-CCACAGTGGAGCCTCCGTTTACCAACTC-3′-TAMRA. Ribosomal 18S RNA was measured for each sample as an internal reference (Applied Biosystems) [22].

Statistical analysis

All experiments were performed two or three times in triplicate, and results are expressed as means ± S.E.M. Statistical significance was determined using Student's t test or ANOVA with Dunnett's post-test comparison. Statistical significance was taken as $P < 0.05$.

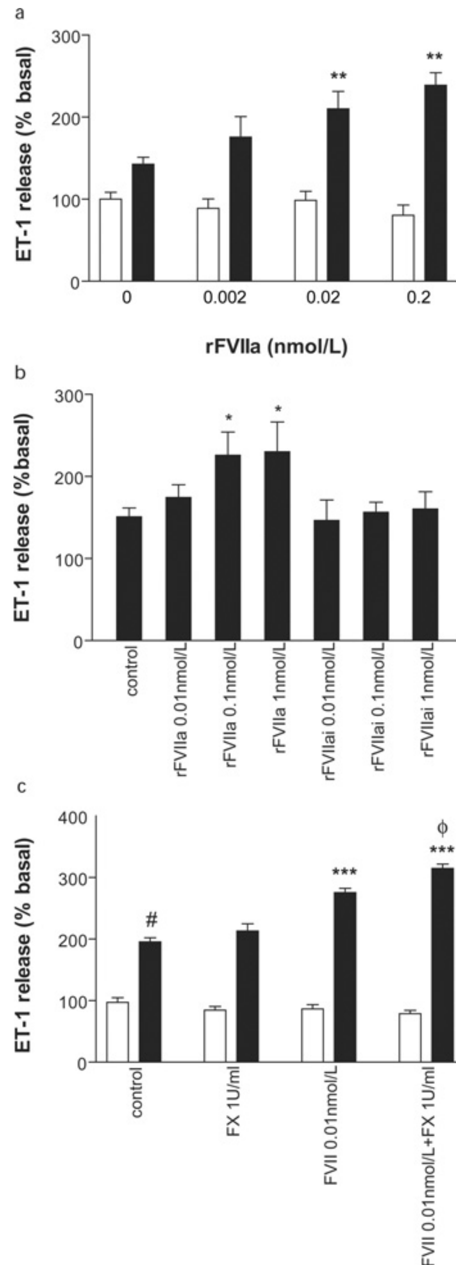

Figure 1 Effect of TNF and FVII on ET-1 release
Black bars indicate TNF-pretreated cells (10 ng/ml), and white bars indicate medium alone. (a) Concentration-dependent effect of rFVIIa on ET-1 release. $^{**}P < 0.01$ compared with TNF alone. (b) The effect of active-site inhibited rFVIIa (rFVIIai) on ET-1 release. $^{*}P < 0.05$ compared with TNF alone. (c) The effect of FVII and FX on ET-1 release. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with TNF alone. $\#P < 0.05$ compared with basal (time 0). $\Phi P < 0.05$ compared with TNF + FVII.

RESULTS

Effect of TNF and FVIIa on ET-1 release

As shown in Figure 1(a), treatment with rFVIIa caused a concentration-dependent increase in ET-1 release from

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TNF-pretreated BAEC. Without TNF pretreatment, rFVIIa had no effect. Inactivation of rFVIIa with *H*-D-Phe-Phe-Arg-chloromethylketone, a specific active-site inhibitor, completely blocked the responses to rFVIIa (Figure 1b), showing that this effect is dependent on the proteolytic activity of rFVIIa.

To investigate whether other coagulation factors could induce ET-1 release, we compared the effects of FVII, FX and FXa on TNF-pretreated cells. FVII significantly increased ET-1 release from cells pretreated with TNF (Figure 1c) with a maximum activity at 0.01 nmol/l. This is consistent with FVII binding to TF on the cell surface and becoming activated to FVIIa. The TF/FVIIa complex converts FX into FXa. FX alone had no greater effect than TNF treatment alone (Figure 1c). The combination of FVII with FX (1 unit/ml) significantly increased ET-1 release compared with FVII alone (Figure 1c). Confirmation that FXa was mediating this increase was obtained by stimulating TNF-treated BAEC with FXa. This significantly increased ET-1 release with a maximum response at 0.01 unit/ml (results not shown), but the response with FXa alone was less than with rFVIIa. Therefore the TF/FVIIa or TF/FVIIa/FXa complexes can both induce this response, and our data show that the TF/FVIIa/FXa complex was the most effective of these stimuli at inducing ET-1 release.

TF function and mRNA expression

As TF is the ligand for FVII on the endothelial cell surface, we investigated whether the response was linked to changes in TF expression. TF activity reached a maximum at 4 h and remained significantly elevated for 6 h (Figure 2a). TF mRNA levels were greatest at 2 h after TNF stimulation (Figure 2b) and then returned towards basal levels. This is consistent with the FVII response being TNF-dependent, as it required the expression of TF for conversion into its activated state.

Effect of PAR agonists and antagonists on ET-1 release and mRNA expression

We investigated the role of PAR2 in FVII-induced ET-1 synthesis. The PAR2 agonist SLIGKV caused a concentration-dependent increase in ET-1 release after pretreatment with TNF (Figure 3a). SLIGKV (100 μmol/l) caused the largest increase, but it was not significantly different from the response at 0.01 μmol/l. This effect was only present after TNF pretreatment. Thrombin and the PAR1-activating peptide SFLLRN also increased ET-1 release. The responses under basal conditions and after TNF pretreatment were not different and the magnitude of the thrombin and SFLLRN responses were similar (Figure 3b). The PAR2 agonist response and rFVIIa response after pretreatment with TNF was significantly greater than the response to the PAR1 agonist SFLLRN or thrombin (Figure 3b). Consistent with the studies of ET-1 release, rFVIIa and SLIGKV significantly increased

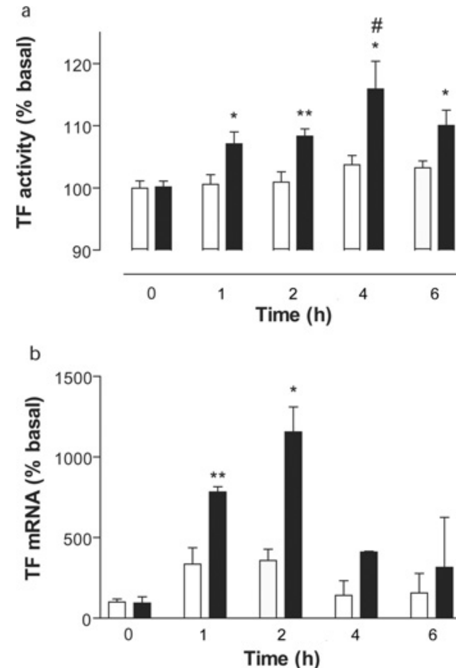

Figure 2 Effect of TNF on TF activity and mRNA expression
Black bars indicate TNF treatment, and white bars indicate medium alone. (a) Time course for TF. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with basal (time 0). $\#P < 0.05$ compared with control (medium alone) at 4 h. (b) Time course for TF mRNA expression after TNF stimulation. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with basal (time 0).

mRNA levels, but only after pretreatment with TNF (Figure 3c). SLIGKV tended to increase ET-1 release and mRNA levels on its own, but this did not reach significance when compared with control levels (Figures 3b and 3c). Thrombin and SFLLRN increased mRNA levels under basal conditions and after TNF treatment, but there was no further augmentation by TNF.

To determine whether PAR2 mediated the response to rFVIIa we tested the effect of receptor desensitization by pretreating with TNF and SLIGKV in combination. This resulted in complete suppression of the responses to subsequent stimulation with FVIIa or further stimulation with SLIGKV, strongly indicating that PAR2 mediated the rFVIIa response (Figure 3d). Desensitization was specific to PAR2 agonist responses as the effect of thrombin and SFLLRN were unaffected.

Analogues of the PAR2-activating peptide have been described that inhibit trypsin-induced activation of PAR2 [23]. Therefore, to confirm the role of PAR2 in the response to FVIIa, we tested the effect of the putative PAR2 antagonist peptide FSLLRY on PAR2 agonist- and rFVIIa-induced ET-1 release. The PAR2 agonist response was fully inhibited (Figure 3e) by FSLLRY, confirming that it was an effective PAR2 antagonist on endothelial cells. Similarly, FSLLRY blocked the response to rFVIIa from 0.003 to 0.1 nmol/l (Figure 3f), showing that PAR2 mediates the interaction between activation of FVII and

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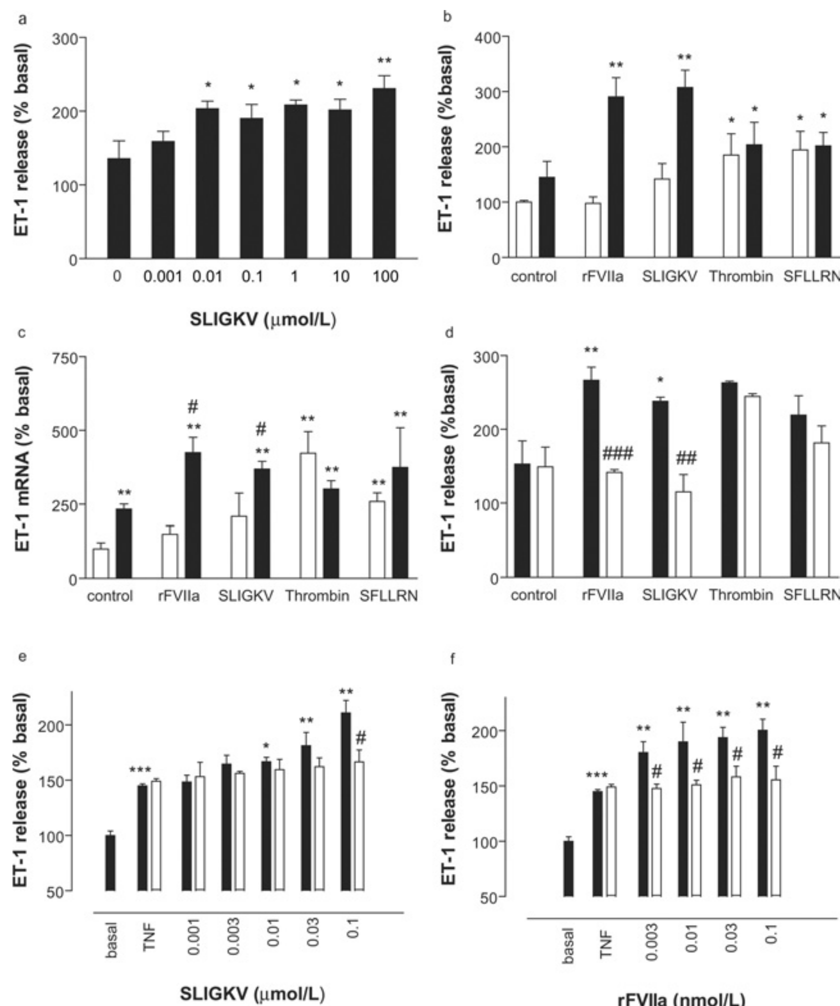

Figure 3 Effect of PAR agonists and antagonists on ET-1 release and mRNA expression

(a) Concentration-dependent effect of PAR2 agonist SLIGKV on TNF-pretreated cells. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with TNF alone. (b) The effect of PAR2 agonist (100 μmol/l) after pretreatment with TNF (black bars) or medium alone (white bars) compared with the effect of thrombin (4 units/ml) and PAR1 agonist (100 μmol/l SFLLRN). $^{*}P < 0.05$ compared with basal; $^{**}P < 0.01$ compared with TNF alone. (c) Effect of treatment for 2 h with rFVIIa (0.2 nmol/l) and PAR agonists (100 μmol/l) on ET-1 mRNA levels after pretreatment for 2 h with TNF (black bars) or medium alone (white bars). $^{**}P < 0.001$ compared with basal. $\#P < 0.01$ compared with TNF alone. (d) Effect of PAR2 desensitization: comparison of ET-1 responses after pretreatment with TNF alone (black bars) or TNF + SLIGKV (white bars). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with TNF. $\#\#P < 0.01$ compared with TNF + SLIGKV. $\#\#\#P < 0.001$ compared with TNF + rFVIIa. Thrombin and SFLLRN responses were significantly raised compared with basal release ($P < 0.05$; not shown on the graph) and were not significantly reduced by desensitization. (e) Effect of PAR2 antagonist FSLLRY (100 μmol/L; white bars) on SLIGKV response (black bars). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with TNF. $^{***}P < 0.001$ compared with basal. $\#P < 0.05$ compared with treatment with agonist at each concentration. (f) Effect of PAR2 antagonist FSLLRY (100 μmol/l; white bars) on rFVIIa response (black bars). $^{**}P < 0.01$ compared with TNF; $^{***}P < 0.001$ compared with basal; $\#P < 0.05$ compared with treatment with agonist at each concentration.

the endothelium. When the properties of FSLLRY were first described, it was found to block the action of trypsin, but not the response to a PAR2-activating peptide on rat aortic rings [23]. Thus we have demonstrated that, during incubation for 4 h, FSLLRY blocked the action of rFVIIa and SLIGKV. Therefore this peptide antagonist may have greater affinity for the bovine PAR2 than the rat receptor, or it may simply reflect the difference between blocking the acute response of rat aortic rings versus a sustained incubation of cultured cells. FSLLRY alone did not affect ET-1 release, although it may have other effects on endothelial cells.

Effect of p38 MAPK inhibition on rFVIIa and PAR2 agonist response

The p38 MAPK inhibitor SB203580 significantly inhibited the rFVIIa (Figure 4a) and SLIGKV (Figure 4b) responses. However, it was only effective when cells were treated for 30 min prior to TNF pretreatment. If used after TNF pretreatment (Figures 4c and 4d), SB203580

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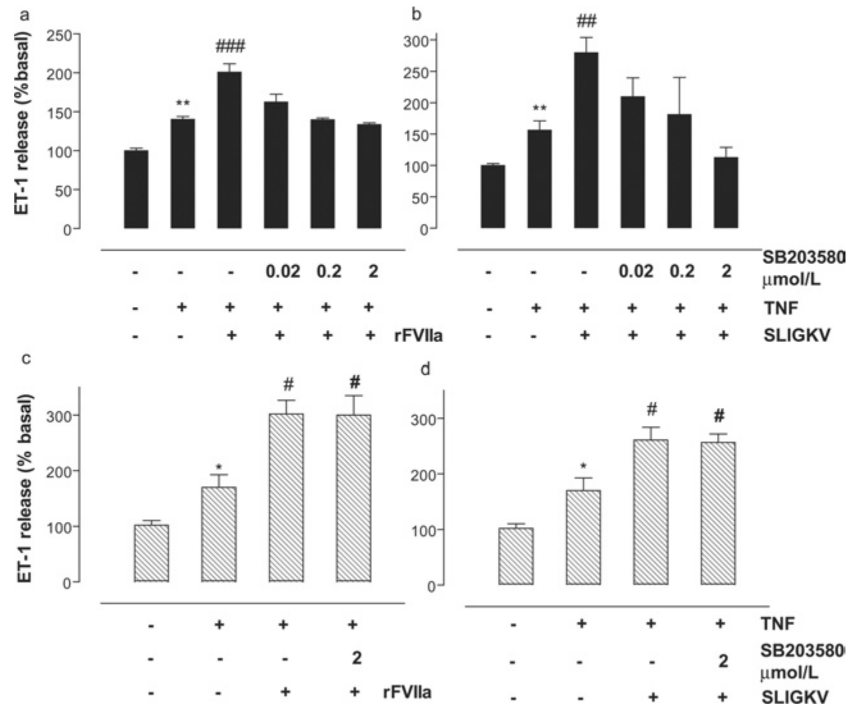

Figure 4 Effect of p38 MAPK inhibition on the rFVIIa and SLIGKV responses
Cells were treated with a p38 MAPK inhibitor (SB203580) either before (a and b) or after (c and d) TNF pretreatment. $*P < 0.05$ and $**P < 0.01$ compared with basal; $\#P < 0.05$, $\#\#P < 0.01$ and $\#\#\#P < 0.001$ compared with TNF.

had no effect on the rFVIIa or SLIGKV responses, indicating that inhibition of p38 MAPK may prevent PAR2 expression.

PAR2 expression and p38 MAPK phosphorylation

To investigate PAR2 expression on endothelial cells further, we performed immunofluorescence staining. Unstimulated cells had a very low expression of PAR2, which was barely detectable (Figure 5a). This basal expression was up-regulated with TNF stimulation (Figures 5b and 5c). Consistent with studies of ET-1 release, treatment of cells with SB203580 before TNF treatment reduced the increase in PAR2 expression (Figure 5d), but did not abolish it completely. This finding suggested that PAR2 expression may be dependent on the p38 MAPK pathway; however, other pathways may also be involved. To confirm the activation of p38 MAPK, we performed immunofluorescence-labelling experiments for phosphorylated p38 MAPK within the nucleus, and TNF treatment increased p38 MAPK phosphorylation compared with basal levels (Figures 5e and 5f).

DISCUSSION

The activation mechanism for PAR2 and its physiological role have not been clearly defined. Earlier studies have proposed FVIIa and FXa as potential activators of PAR2 [18]. Our present results demonstrate that FVII activates PAR2 on TNF-primed endothelial cells, and is potentiated further by FX, with this leading to ET-1 synthesis. The response is dependent on TF expression, which is up-regulated by inflammatory stimuli, and then binds and activates FVII. Traditionally this was viewed as the initiating step for thrombosis, but our present results show that simultaneous activation of PAR2-mediated responses will also occur. This provides a key link between the initiation of thrombosis, inflammation and altered endothelial function.

Although thrombin plays a central role in coagulation and has numerous effects on human vascular biology through its action on PAR1 and PAR3 [17], it does not activate PAR2. There is some evidence for the transactivation of PAR2 by PAR1, but the functional significance of this is unknown [24]. Also, there is some evidence that SFLLRN may not be a specific PAR1 agonist and may activate PAR2 [25]. These experiments were performed by expressing human and murine PAR2 in *Xenopus* oocytes. There is no data available on PAR ligand cross reactivity in BAEC. Although there are reports that SFLLRN may not be highly specific on human and murine receptors, the response to SFLLRN was not affected by desensitization with SLIGKV in our present experiments, which suggests that SFLLRN shows greater selectivity on bovine PAR1. Our data have shown that,

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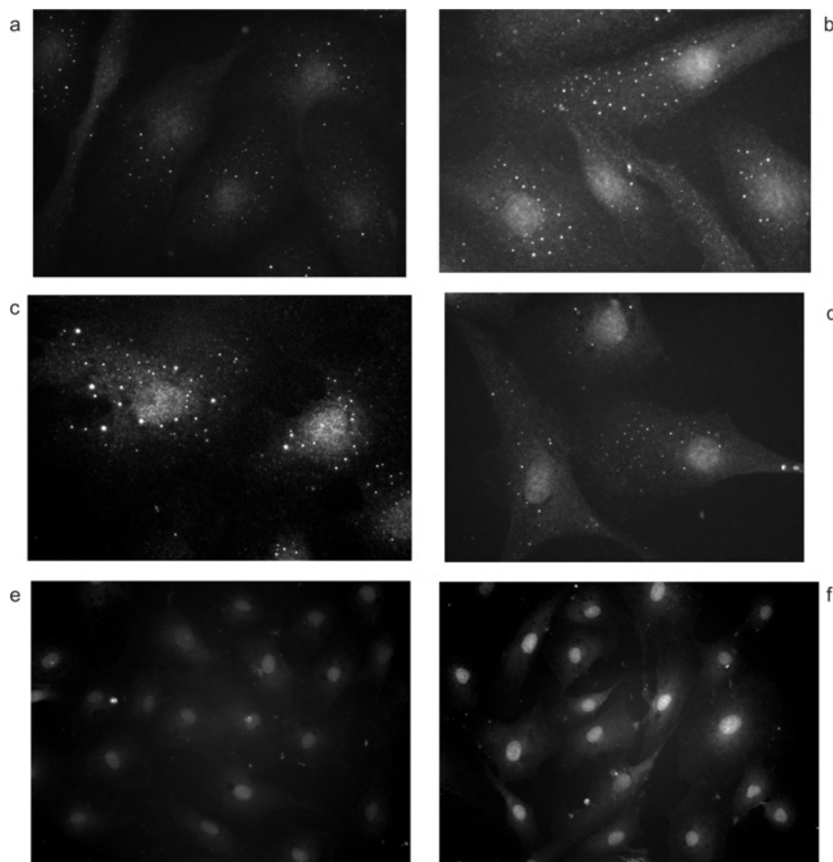

Figure 5 PAR2 expression and p38 MAPK phosphorylation

(a) Immunofluorescence staining for PAR2 under basal conditions (original magnification, × 40). (b) Immunofluorescence staining for PAR2 after TNF treatment showing increased PAR2 expression. (c) PAR2 expression persisted after overnight stimulation with TNF. (d) Inhibition of PAR2 expression by treating with SB203580 before TNF exposure. (e) Immunofluorescence staining for nuclear p38 MAPK phosphorylation showing basal levels. (f) p38 MAPK phosphorylation increased with TNF treatment.

although thrombin does increase ET-1 synthesis, the response is not affected by pretreatment with TNF. Thrombin-induced ET-1 release has been well described [26].

In the present study, we have demonstrated that PAR2 can be activated either directly by a synthetic peptide or by FVII. As yet, the endogenous ligand for PAR2 is not known. Therefore PAR2 may function as a receptor for sensing the presence of both inflammation and the initiation of thrombosis within the vasculature. This is likely to be of major importance both in atherogenesis preceded by a prothrombotic state and in the inflammatory environment of an unstable plaque. This is emphasized by the fact that deficiency in the TF pathway inhibitor, which regulates the extrinsic pathway of coagulation by inhibiting the TF/FVIIa/FXa complex, promotes atherosclerosis and thrombosis [27]. Importantly, the ET-1 response to FVIIa was elicited with concentrations used within the physiological range (0.02 to 0.2 nmol/l), which supports the concept that FVII is a key endogenous regulator of PAR2. Previous studies have examined the effect of much higher concentrations of FVIIa on endothelial cells (50 nmol/l), but these studies used HUVEC (human umbilical vein endothelial cells) and found little response on phosphoinositide hydrolysis [18]. The difference in response to that found in our present study may be related to different cell types as we used aortic endothelial cells. Also, different culture conditions, non-physiological concentrations of FVIIa and different end results were measured in the previous study [18]. A recent study has shown that SLIGKV can cause endothelium-dependent NO-mediated vasodilatation in healthy volunteers [28]. Therefore endothelial phenotype probably has the greatest influence on the response to PAR2 agonists and this may depend on the degree of PAR2 expression, which will differ in sepsis, coronary artery disease and healthy subjects.

It is well established that atheroma-prone sites in the vascular tree are localized to points of turbulent blood flow where there are marked gradients in shear stress [29]. Consistent with this, TF expression is suppressed by chronic physiological levels of shear stress [30]. However, acute changes in shear stress up-regulate TF both *in vivo* and *in vitro* [31], inducing a prothrombotic phenotype

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exemplified by the characteristics of the endothelium at atheroma-prone sites such as the carotid bifurcation [32]. This is also seen in the present experiments with the up-regulation of TF mRNA in untreated cells, which is most probably related to the non-laminar shear stress of changing culture medium at 2 and 4 h. The consequent binding and activation of FVII and FX on the endothelium in these areas would therefore be expected to induce ET-1 synthesis through the PAR2-dependent mechanism described in the present study and hence drive the process of atheroma formation.

Therefore treatments that prevent PAR2 activation have potential for preventing atherogenesis and reducing ischaemia in acute coronary syndromes, when ET-1 synthesis is driven through FVII-dependent mechanisms. PAR2 activation stimulates stress-activated protein kinases [33]. However, we have found in the present study that treatment with SB203580 reduced cell-surface expression of PAR2, although this was not quantified. This shows that the p38 MAPK pathway may be important for the regulation of PAR2 surface expression induced by inflammatory stimuli. Recently, SB203580 has also been reported to inhibit TNF-induced expression of TF in endothelial cells [34]. Hence the effects of p38 MAPK inhibition on FVIIa responses could be due to a combined lack of TF and PAR2. Therefore the modulation of PAR2 surface expression may provide another therapeutic target for treatment of coronary artery disease.

High levels of circulating TF contribute to the increased thrombotic complications associated with the presence of cardiovascular risk factors, particularly diabetes, smoking and hyperlipidaemia. In these conditions, the prothrombotic state will lead to ET-1 synthesis via FVII-dependent activation of PAR2. This provides a much sought explanation for the link between prothrombotic states and vascular dysfunction. Modulation of this response by PAR2 antagonists will be useful in the prevention of atherogenesis and in the treatment of acute coronary syndromes.

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