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Factor VIIa binding to endothelial cell protein C receptor

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Abstract

Recent studies have shown that factor VIIa (FVIIa) binds specifically to endothelial protein C receptor (EPCR), a known cellular receptor for protein C and activated protein C, on the endothelium. The formation of FVIIa:EPCR complexes neither supports the activation of coagulation nor modulates tissue factor-initiated coagulation. However, FVIIa interaction with EPCR, particularly at pharmacological concentrations of FVIIa, may impair EPCR-dependent protein C activation and activated protein C-mediated cell signaling by competing directly with them for binding to EPCR. FVIIa binding to EPCR may also contribute to FVIIa clearance. This review summarizes recent data on FVIIa interaction with EPCR and discusses potential physiological significance and consequences of the interaction.

Keywords

Factor VIIa; Endothelial cell; protein C receptor; Endothelium

Introduction

Endothelial cells that line the lumen of the blood vessels provide the interface between the vessel-wall tissue and the circulating blood. In a quiescent state, endothelium provides a non-thrombogenic surface by lacking the expression of procoagulant receptor, tissue factor (TF) and expressing receptors that are crucial for the anticoagulant pathway, such as endothelial cell protein C receptor (EPCR). By being at the interface between the blood and the vessel wall, endothelial cells are optimally positioned to interact with circulating clotting factors; however, there is little information on how clotting factors might interact with the endothelium, or what the consequences of any interaction might be. In this article we review studies on the interaction of factor VIIa (FVIIa) with endothelial cells, specifically FVIIa binding to EPCR on the endothelium. We also speculate on the potential role/effects of this interaction.

FVIIa interaction with endothelial cells

In 1984 Rodgers et al. [1] analyzed ¹²⁵I-FVII binding to intact cells derived from vascular and non-vascular tissues and correlated the level of FVIIa binding with the procoagulant activity of these cells. Their data revealed that only non-vascular cells expressing TF possessed receptors for FVII. Other cells derived from vascular tissues, such as aortic endothelial cells, did not. Further, the cells with the highest amount of procoagulant and TF activity possessed the most receptors for FVII. These data were consistent with the well-accepted belief that TF

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Conflict of interest statement

The authors have no conflicts of interest.

is the only receptor for FVII/FVIIa, and that FVII/FVIIa does not associate with unperturbed endothelial cells as they lack TF on their cell surfaces. However, recent research has brought this paradigm into question. Reuning et al. [2] found that FVII/FVIIa bound to both stimulated as well as unstimulated human umbilical vein endothelial cells (HUVEC), and that only 10% FVII/FVIIa binding to stimulated cells is blocked by anti-TF antibodies, suggesting that FVIIa can bind to endothelium at two distinct sites, one being the TF on stimulated endothelial cells. The second site, present on both stimulated and unstimulated HUVEC, appeared to be a common binding site for vitamin K-dependent proteins since prothrombin and other vitamin K-dependent proteins blocked the FVIIa binding to endothelial cells. FVII/FVIIa binding to this common site would be of minor physiological significance because the concentration of FVII in plasma is miniscule compared to the concentrations of other vitamin K-dependent proteins, particularly prothrombin, and consequently little if any FVII would bind to the endothelium. Because of this, the study failed to garner enough interest to stimulate studies on identification of the putative receptor on the endothelium involved in FVIIa binding.

To identify potential clearance mechanisms of FVII/FVIIa from the circulation, we recently investigated the binding and internalization of FVIIa in various cell types, including native endothelial cells. Studies with unstimulated HUVEC showed that FVIIa associates with endothelial cells in a time-, temperature- and dose-dependent manner. The binding is calcium dependent but independent of TF and negatively charged phospholipids [3]. The N-terminus domain that contains γ -carboxyglutamic acid (Gla) residues is essential for FVIIa binding to endothelial cells. Interestingly, the binding of ^{125}I -FVIIa to endothelial cells is blocked by a molar excess of unlabelled factor X or protein C but not by prothrombin or factor IX. These data suggested, contrary to the earlier report [2], that the FVIIa binding site on unstimulated HUVEC is not a common binding site for all vitamin K-dependent protein but that FVIIa appears to share one or more binding sites with protein C and factor X. Although there was no information in the literature on specific factor X binding sites on the endothelial cells, EPCR is a well-characterized receptor for protein C/APC on endothelial cells [4].

FVIIa binding to EPCR

EPCR, first cloned and characterized by the Esmon laboratory in the early 1990s, is primarily expressed by the vascular endothelium, preferentially on the endothelial cells of large blood vessels [5]. Recent studies have shown expression of EPCR in other cell types, including monocytes [6] and hematopoietic stem cells [7]. EPCR binds to protein C and increases the rate of protein C activation by thrombin:thrombomodulin complexes [8]. EPCR has been shown to play a role in hematopoiesis, autoimmunity, and the control of both the coagulation and inflammation responses to infection and trauma [9]. Recent studies have also suggested that APC bound to EPCR could initiate protease-activated receptor-1 (PAR-1)-mediated cell signaling [10] and that this activation may be responsible for the various anti-inflammatory activities that APC and EPCR seem to stimulate [11], such as the prevention of apoptosis [12] and protection of the endothelial cell barrier [13].

The observation that a monoclonal anti-EPCR antibody blocked ^{125}I -FVIIa binding to HUVEC is consistent with the hypothesis that FVIIa binds to EPCR on endothelial cells. FVIIa binding to EPCR was further confirmed by the demonstration of a several-fold increase in ^{125}I -FVIIa binding to CHO cells that had been transfected with an EPCR-expressing plasmid compared to the wild-type CHO cells. The increased ^{125}I -FVIIa binding to CHO/EPCR cells was markedly reduced by the addition of protein C, and completely attenuated by an anti-EPCR antibody. It is important to note that unlike the data obtained with HUVEC, a 100-fold molar excess of unlabelled factor X failed to block ^{125}I -FVIIa binding to EPCR-expressing CHO cells. This rules out the possibility that factor X also binds to EPCR. It is interesting to note that both FVII and FVIIa bind to EPCR on non-stimulated HUVEC with a similar affinity (Kd,

~30–50 nM range) as that of protein C/APC [14]. The number of EPCR-specific FVII/FVIIa binding sites on endothelial cells (~50,000 sites/cell) is also very similar to the number of protein C/APC binding sites. These findings indicate that EPCR acts a true receptor for FVII/FVIIa. Since FVII and protein C bind to EPCR with similar affinities, the amount of each ligand associated with the EPCR would be approximately proportional to their respective concentrations.

In parallel to our studies on FVIIa binding to EPCR on endothelial cells, Preston et al. [15], while characterizing the protein C Gla domain interaction with EPCR using BIAcore, also found that FVIIa binds to EPCR. Protein C binding to EPCR is mediated by the Leu-8 residue in the ω -loop of the Gla domain [15], and this binding region is completely conserved in FVII. An alanine mutant scanning approach with EPCR showed that the residues of EPCR previously identified as being directly involved in the interaction with protein C/APC [16] are also involved in the binding of FVII/FVIIa [17].

Cellular consequences of FVIIa binding to EPCR

Unlike the situation with FVIIa binding to TF, FVIIa binding to EPCR has little effect on FVIIa catalytic activity. On unstimulated endothelial cells, EPCR:FVIIa complexes activate neither factor X nor the protease activated receptors, PAR1 or PAR2. This suggests that EPCR does not induce the necessary essential allosteric conformational changes in FVIIa to enhance its proteolytic activity [3]. However, Lopez-Sagasetta et al. [17] reported a noticeable influence of sEPCR on the amidolytic activity of FVIIa, suggesting that the molecule does undergo a structural rearrangement involving its active site. It is interesting to note that sEPCR increased the amidolytic activity of FVIIa even when FVIIa was complexed with TF [17]. These data need to be confirmed. In our opinion, FVIIa binding to EPCR on stimulated endothelial cells would have little effect on the procoagulant activity of the activated endothelium because FVII/FVIIa binds to TF with much higher affinity than it binds to EPCR. Thus the binding of FVIIa to EPCR will not have a significant influence on the formation of TF:FVIIa complexes on activated endothelial cells. In light of this, a recent observation that showed blocking of the EPCR binding site on EA.hy926 cells increased the generation of FXa at low concentrations of FVIIa [17] is surprising. However, we are unable to reproduce these data with stimulated HUVEC (unpublished data).

At present, the physiological importance of FVII/FVIIa binding to EPCR remains unclear because FVII circulates in plasma at a 7-fold lower concentration than protein C and both molecules bind to EPCR with a similar affinity. Thus it is unlikely that FVII acts as the major ligand for EPCR under normal physiological conditions. However, under therapeutic conditions, FVIIa levels may be elevated close to protein C levels in blood, and so it may compete with protein C to bind to EPCR. Consistent with this scenario, therapeutic concentrations of FVIIa have been shown to significantly, but not completely, inhibit thrombin thrombomodulin-mediated APC generation in cell model systems [3,17]. Thus it is conceivable that therapeutic concentrations of FVIIa can down-regulate the APC-mediated anticoagulant pathway *in vivo* by competing with protein C/APC for EPCR, and this could contribute to the therapeutic outcome of rFVIIa therapy namely the cessation of hemorrhage.

Although it is tempting to speculate that FVIIa binding to EPCR can induce cell signaling in endothelial cells akin to APC binding to EPCR, we found no evidence in our initial studies that EPCR:FVIIa activates PAR1 or PAR2, as measured by its ability to cleave PARs or induce phosphorylation of p44/42 MAPK in CHO cells transfected with EPCR [3]. Recent studies of Bae et al. [18] also suggested that FVIIa binding to EPCR does not induce cell signaling since FVIIa failed to prevent enhanced cell permeability induced by thrombin. However, these studies alone do not completely rule out a role for EPCR-FVIIa in cell signaling.

The relevance of FVII/FVIIa binding to EPCR may not be in modulating the activities of FVIIa, APC or EPCR-mediated cell signaling, but possibly in the clearance of FVII/FVIIa from the circulation. Our studies showed that FVIIa was internalized by both cultured endothelial cells and EPCR-transfected CHO cells [3]. The internalization was mediated by EPCR as EPCR mAb reduced the extent of internalization. However, at present it is unclear whether EPCR-mediated FVIIa endocytosis occurs *in vivo*, and if it does, whether it is rapid or significant enough to regulate FVII/FVIIa levels in circulation. Experiments are underway in our laboratory to test this hypothesis.

Summary

Recent *in vitro* studies provide unequivocal evidence for FVII/FVIIa binding to EPCR. At present it is unclear whether this interaction has any physiological relevance, but it certainly has a potential to modulate the protein C-mediated anticoagulation pathway and FVII/FVIIa clearance. Further studies are required, particularly studies using appropriate animal model systems, to evaluate the importance of the interaction of FVIIa with EPCR in pathophysiology.

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