Is EPCR a multi-ligand receptor? Pros and cons

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Summary
In the last decade, the endothelial cell protein C/activated protein C receptor (EPCR) has received considerable attention. The role initially attributed to EPCR, i.e. the enhancement of protein C (PC) activation by the thrombin-thrombomodulin complex on the surface of the large vessels, although important, did not go beyond the haemostasis scenario. However, the discovery of the cytoprotective, anti-inflammatory and anti-apoptotic features of the activated PC (APC) and the required involvement of EPCR for APC to exert such actions did place the receptor in a privileged position in the crosstalk between coagulation and inflammation. The last five years have shown that PC/APC are not the only molecules able to interact with EPCR. Factor VII/VIIa (FVII/VIIa) and factor Xa (FXa), two other serine proteases that play a central role in haemostasis and are also involved in signalling processes influencing wound healing, tissue remodelling, inflammation or metastasis, have been reported to bind to EPCR. These observations have paved the way for an exploration of unsuspected new roles for the receptor. This review aims to offer a new image of EPCR in the light of its extended panel of ligands. A brief update of what is known about the APC-evoked EPCR-dependent cell signalling mechanisms is provided, but special care has been taken to assemble all the information available about the interaction of EPCR with FVII/VIIa and FXa.

Keywords
EPCR, protein C, factor VII, factor X, endothelium

Introduction: the identification of EPCR as a receptor for PC/APC

Endothelial cell protein C/activated protein C receptor (EPCR) was initially described as an endothelial transmembrane glycoprotein able to bind to protein C (PC) and activated protein C (APC) with high affinity (Kd = 30 nM) in a Ca2+-dependent manner (1). This, and the immediately subsequent work performed by Dr. Esmon’s group, showed that EPCR belonged to the CD1/major histocompatibility complex superfamily, that it was reasonably conserved between species, which suggests a prominent functional role, and that its expression was abundant in the endothelium of the large vessels and in the trophoblast (2–5).

The remarkable increase in APC generation by the thrombin-thrombomodulin complex when EPCR was present constituted the first evidence of its functional relevance (6). EPCR is predominantly located in the lipid rafts of the membrane where it colocalises with thrombomodulin (7). PC binds to EPCR through its Gla domain (8). As a result, PC is far more accessible to thrombomodulin-bound thrombin and the efficiency in the generation of APC is improved around 10-fold in vivo, which is a result of a decrease in the apparent Km for activation (9). Blocking EPCR helped to accelerate vessel occlusion in a murine thrombosis model (10). A blocking anti-EPCR autoantibody was found in a patient who had experienced multiple venous thrombosis episodes (11). Accordingly, anti-EPCR autoantibodies were found to be associated with higher thrombotic risk in diverse situations (11–13). These findings underline the notable anticoagulant role played by EPCR in vivo. Nevertheless, it has been suggested that low expression levels would be enough to accomplish such a function (14). On the other hand, some polymorphisms of the EPCR gene, one of which is known to influence around 10% of the plasma levels of protein C (15, 16), could influence the thrombotic risk to some extent, although the literature on this issue is highly controversial (15, 17–27, reviewed in [28, 29]).

The apparent success of APC in reducing mortality in patients with severe sepsis (PROWESS study) in spite of the failure achieved by other anticoagulant strategies (30–32), together with the finding that blocking EPCR exerts devastating effects upon subthal endotoxin administration (33), suggested that the interaction between APC and EPCR could also trigger relevant protective cell signalling mechanisms. Since then, numerous studies have de-
scribed the beneficial consequences of such an interaction in a variety of inflammatory diseases. The EPCR-dependent effects of APC have been the topic of some excellent recent reviews (34–36). For this reason, we are going to briefly summarise the most relevant findings, and then focus our attention on the less widely explored field of the interaction of EPCR with factor (F)VII/VIIa and FXa.

**APC induces antiapoptotic, antiinflammatory and barrier-protective effects upon interaction with EPCR**

Shortly after the PROWESS study trial had been presented, a profile was obtained of the endothelial genes whose expression could be modulated by the interaction between APC and EPCR. It was not surprising that a group of probes corresponding to genes involved in inflammation, cell adhesion or apoptosis were among those that were most highly regulated (37). Further studies confirmed these results and provided new information about other genes whose expression was altered upon APC-EPCR interaction, always favouring antiadhesive and antiapoptotic patterns (38–44). These actions were nicely summarised by Mosnier et al. (45).

Protease activated receptor-1 (PAR-1), a G-protein coupled receptor which is present in the lipid rafts of the cell membrane, was shown to be activated by APC when the latter was bound to EPCR. The subsequent PAR-1 signalling accounted for the APC-induced protective gene expression profile (38). Not only the exogenously administered APC but, remarkably, even the endogenous PC activation on the endothelial surface is mechanistically linked to the PAR1-dependent protective effects (46). PAR-1 was known as the receptor engaged by thrombin to trigger cell signalling mechanisms leading to proinflammatory, proadhesive, barrier disrupting and propapoptotic actions (47). The affinity of thrombin for PAR-1 is several orders of magnitude higher than that exhibited by APC (48) and thrombin is

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**Figure 1: The role of EPCR in the regulation of coagulation.**

A) The binding of PC to EPCR facilitates the interaction of the former with the thrombomodulin-bound thrombin. As a result, the generation of APC and the subsequent APC-dependent inactivation of factors Va and VIIIa is greatly increased. B) The binding of FVII to EPCR prevents the colocalisation of the former with FXa on the cell membrane phospholipids. As a result, the FXa-dependent activation of FVII is reduced. Indeed, since PC and FVII bind to EPCR with similar affinity, both species act as inhibitors of each other in the aforementioned actions. The ellipses in PC, APC, FXa, FVII, and FVIIa represent their Gla domains.
For these reasons it is not totally understood how the endogenously generated APC is able to induce PAR-1-dependent mechanisms, and why such mechanisms are opposite to those exerted by thrombin when both of them activate the same mediator (42). Although further confirmatory evidence is needed, a plausible explanation, which is supported by experimental evidence (49–53), has been suggested (Fig. 2): EPCR, when not occupied by a ligand, may remain associated with caveolin-1, which is also present in the rafts. Under these conditions the thrombin-dependent activation of PAR-1 would elicit a proinflammatory signal through $G_{12/13}$ and $G_{q}$, and an antiinflammatory expression pattern would be triggered. The protective signalling activity of thrombin would reverse to its classical proinflammatory one only when thrombin reached a concentration high enough to activate PARs other than PAR-1 (54). Very recently, the observation that a thrombin derivative containing the Gla-domain of APC was protective against murine sepsis by exerting a superior inhibition, when compared to APC, of the high mobility group box 1 signalling in endothelial cells, has pro-

![Figure 2: The fate of the thrombin-dependent PAR-1 signalling may be influenced by the occupancy of EPCR by its ligands.](image-url)
vided additional support for this theory (55). However, other options remain open. A recent study has involved β-arrestin recruitment and subsequent activation of dishevelled-2, instead of G i coupling, to orchestrate the anti-inflammatory actions of APC-activated PAR-1 (56). On the other hand, experiments performed on the surface of endothelial cells using thrombin and APC simultaneously showed that, while thrombin-activated PAR-1 was rapidly internalised and degraded, APC-activated PAR-1 remained on the cell surface. This would mean that although the rate of thrombin-PAR1 cleavage might be much higher, the APC-cleaved receptor would accumulate on the surface and could thus mediate relevant signalling even in the presence of thrombin (57). Nevertheless, why APC recruits PAR-1 to protective signalling would remain unexplained.

The APC-EPCR-PAR-1 axis has been proven to reduce organ damage in models of stroke, ischaemic injury, sepsis and autoimmune disease. Particularly promising is the role that pharmacologically administered APC may play in the near future in stroke treatment, as shown in several murine stroke models. A clinical trial examining the usefulness of APC treatment in ischaemic stroke is currently being carried out (clinical trial identifier NCT00533546; http://www.clinicaltrials.gov). APC was primarily shown to exert anti-inflammatory, antithrombotic, and neuroprotective effects in a model of focal ischaemic stroke (58). Subsequently, it was shown that in human brain endothelium, APC regulated the intracellular Ca 2+ concentration through the EPCR-PAR-1 axis (59) and inhibited apoptosis by reducing the expression of tumor suppressor protein p53, normalising the pro-apoptotic Bax/Bcl-2 ratio and reducing the caspase-3 signalling (40). Furthermore, APC extended the time window of tissue-plasminogen activator (t-PA)-dependent thrombolytic therapy (60). APC blocked the t-PA-induced apoptosis and the nuclear factor kappa B-dependent metalloprotease-9 (MMP-9) pathway in ischemic brain endothelium (61, 62). The exacerbated activity of MMP-9 leads to the loss of integrity of the blood brain barrier and accounts for the non-desired prohaemorrhagic side effect of t-PA. Thus, the EPCR-dependent APC actions would contribute not only to reducing the lesion size but, interestingly, to reducing the t-PA-induced brain haemorrhage (62). The antiapoptotic effects of APC have also been shown to protect against diabetic nephropathy by acting on endothelial cells in an EPCR and PAR-1-dependent manner, and on podocytes via PAR-3 (63, 64).

The APC-dependent reinforcement of the endothelial barrier has been widely reported (65–68). Strikingly, APC may be thought of as an antimetastatic agent since it has been demonstrated that endogenous APC limits cancer cell extravasation through vascular endothelial barrier enhancement, in an EPCR and PAR-1-dependent process which involves phosphorylation of the sphingosine-1-phosphate receptor 1 (69, 70). The upregulation of Tie2, the Angiopoietin-1/Angiopoietin-2 ratio and the tight junction proteins zonula occludens-1 and VE-cadherin would be ultimately responsible for the barrier enhancement (67, 70).

The important role played by EPCR in the host defense against infections was reported shortly after its discovery (33). As stated above, APC initially succeeded in reducing severe sepsis-associated mortality (30), and until recently it has been the first and only drug approved by the Food and Drug Administration (FDA) for the treatment of severe sepsis. During the preparation of this manuscript the company that owns the rights to APC for clinical use has withdrawn it from the market, since an as yet unpublished study has failed to demonstrate a survival benefit of treating septic shock patients with this drug. Nevertheless, signalling of the exogenously administered or endogenously generated APC has been shown to be critical in protection from lipopolysaccharide (LPS)-induced septic shock or acute lung injury in animal models (71–74). The non-hematopoietic, i.e. endothelial, EPCR is required for the regulation of the coagulation and inflammatory responses during endotoxaemia (75). Mice with a severe deficiency of EPCR showed haemodynamic and cardiac alterations, and lower survival upon LPS challenge (76), although it must be noted that another study showed that the lack of one of the two EPCR alleles had little influence on the response to LPS in spite of a 30% decrease in the generation of APC (77).

APC has been used with success in other inflammatory diseases. Its administration was therapeutically effective in ameliorating experimental colitis in mice in an EPCR-dependent manner (78). It may also be of benefit in the control of Dengue shock syndrome (79). Finally, very recently the usefulness of APC for the treatment of systemic lupus erythematosus (SLE) has also been suggested after it was reported that APC succeeded in suppressing the abnormal systemic immune activation in SLE mice, probably through both EPCR-dependent and EPCR-independent mechanisms (80).

All the above referenced actions do not require the anticoagulant activity of APC. In fact, one of the limitations of APC when thought of as a drug is the concomitant haemorrhage risk, which is indeed a current major limitation in its clinical use (81). Recently, APC has been engineered to lose its anticoagulant activity while preserving its cytoprotective properties (82–84). Indeed, the preservation of the EPCR binding ability was a necessary requisite. The mutants have proven to be as useful as their wild-type counterparts in endotoxaemia and stroke models, thus emerging as a therapeutical tool for the near future ([85–90], reviewed in [91]). The recent failure of APC to improve survival in septic shock patients (unpublished results) could be partially related to the administered doses, which should be managed carefully in order to prevent the concomitant bleeding risk. It is tempting to speculate that cytoprotective-only APC variants could be administered at higher doses, thus improving the clinical efficiency.

The EPCR-PC/APC complexes have been shown to internalise into the cytoplasm of the endothelial cells. The receptor-ligand complexes accumulate in a recycling compartment before being targeted back to the cell surface. This mechanism is not used to deplete the membrane of EPCR since the endocytosed EPCR recycles back fully to the cell surface. However, it may facilitate the transcytosis of PC/APC from the apical to the basal side of the cell thus helping to clear it from the circulation and reach the extravascular space (92). These findings are of significance because they strongly suggest that endothelial EPCR facilitates APC to exert cell signalling actions outside the vessel lumen. In fact, the EPCR-assisted transport of APC across the mouse blood-brain barrier has recently been demonstrated (90). Figure 3 summarises the effects...
evoked by the interaction between EPCR and APC or PC on the endothelial surface.

EPCR is also abundantly present on the membrane of CD8+ dendritic cells, and its interaction with APC has proven to be relevant: a recent study has shown that not only is the non-hematopoietic EPCR critically involved in the APC-dependent protective mechanisms during endotoxemia, but that CD8+ dendritic cells are also required mediators for APC to prevent mortality (86).

EPCR is expressed in other cell types, albeit at a lower level. Nevertheless, APC has been shown to trigger EPCR-dependent cell signalling effects on monocytes (93–95), lymphocytes (96), neutrophils (97), gastric epithelial cells (98), keratinocytes (99–102), tenocytes (103), chondrocytes (104), osteoblasts (105), cardiomyocytes (106), and vascular smooth muscle cells (VSMC) (107).

Structural basis of the interaction between EPCR and APC: Clues in the search for new EPCR ligands

Binding between EPCR and PC/APC involves the Gla domain of the latter. The crystal structure of the complex between EPCR and the Gla domain of PC was solved and allowed to identify the resi...
Table 1: Alignment of the regions of the human PC Gla domain involved in EPCR binding with the corresponding Gla domain regions of other serine proteases of the haemostatic system. Bold residues in human PC are directly involved in binding to human EPCR (108). Among them, those conserved in the rest of the molecules have also been marked in bold. Numbering corresponds to the sequence of mature human PC. h, human; m, murine; FII, prothrombin; FIX, factor IX; PS, protein S; PZ, protein Z. NCBI accession numbers are NP_000303.1 for hPC, NP_062562.1 for hFVII, NP_000495.1 for hFX, NP_000497.1 for hFII, NP_000124.1 for hFIX, Z. NCBI accession numbers are NP_000303.1 for hPC, NP_062562.1 for human; m, murine; FII, prothrombin; FIX, factor IX; PS, protein S; PZ, protein Z. Bold residues in regarding its interaction with hEPCR, which will be discussed below. Further, in the human setting, PC activation could be severely compromised in certain situations, for instance in haemophilic or severe trauma patients under antihaemorrhagic treatment with FVIIa, whose plasma levels may reach concentrations around 50 nM or higher (116, 117). The down-regulation of APC generation was found in certain situations, for instance in haemophilic or severe trauma patients under antihaemorrhagic treatment with FVIIa, whose plasma levels may reach concentrations around 50 nM or higher (116, 117). The down-regulation of APC generation will benefit these subjects since the reduction in the APC levels should provide an additional procoagulant effect.

**hFVIIa and Vila are ligands of hEPCR. Functional consequences**

Almost simultaneously, three groups found evidence that hFVIIa Vila were able to bind to hEPCR with high affinity (109, 114, 115). The interaction was very similar to that reported for hPC/APC i.e. it is dependent on the presence of Ca$^{2+}$, improved by Mg$^{2+}$ and defined by a dissociation constant (Kd) around 40 nM, as assessed by diverse approaches like surface plasmon resonance (SPR) or binding kinetics between endothelial cells and fluorescently or radioactively labelled hFVIIa (109, 115). Accordingly, the residues of hEPCR involved in the interaction were also needed for hPC binding (109).

A first functional consequence was that hFVIIa, by preventing hPC to bind to hePCR, dose-dependently reduced the activation of the latter by the thrombin-thrombomodulin complex on the endothelial surface (115). Although at physiological concentrations of both species, 10 and 60 nM, respectively, hPC activation was only slightly modified, and the reduction was close to 50% when both species were at equimolar concentrations (109). Therefore, in the human setting, PC activation could be severely compromised in certain situations, for instance in haemophilic or severe trauma patients under antihaemorrhagic treatment with FVIIa, whose plasma levels may reach concentrations around 50 nM or higher (116, 117). The down-regulation of APC generation will benefit these subjects since the reduction in the APC levels should provide an additional procoagulant effect.

| hPC | ANSFL EELR | EEAKE IF |
| hFVII | ANAFLEELR | EEAREIF |
| hFX | ANSFL EEMK | EEAREVF |
| hFII | ANTFLEEV R | EEA EAL |
| hFII | N SKL EEFV | EEAREVF |
| hPS | ANSSL EETK | EEAREVF |
| hPZ | GSY L EELF | EEAREVF |
| mPC | ANSFL EEMR | EEA EIF |
| mFVII | ANS L L E LW | EEA EIF |
| mFX | ANSF EE FK | EEAREIF |
| mFII | ANSF G L EEL | EEA EAL |
| mPS | NSKG EEFV | EEAREVF |
| mPC | ANTLF EMT | EEAREVF |
| mPZ | GSS YF L EEL | EEAREVF |
In normal haemostasis, there are traces of circulating FXa which activate small amounts of FVII on the unperturbed endothelial surface, and vice versa, i.e. traces of FVIIa which activate small amounts of FX. These activations occur because both species colocalise through binding to traces of negatively charged phospholipids, i.e. phosphatidylserine, exposed on the cell membranes. Such reactions do not proceed very efficiently since the presence of tissue factor (TF) is scarce, but the amounts of FVIIa and FXa so generated contribute to maintain coagulation at a level high enough for day-to-day needs (118). EPCR, by binding FVII and FVIIa, should partly prevent them interacting with phosphatidylserine. Thus, these activating reactions should be in part EPCR-down-regulated. When this hypothesis was tested, hEPCR, by preventing the interaction between hFVII and phosphatidylserine, did reduce significantly the amount of hFVII activated by hFXa on human aortic endothelial cells (HAEC), since a blocking monoclonal antibody (mAb) against hEPCR increased significantly the activation of hFVII. Importantly, hFVII activation was similar in the presence or absence of hPC when both of these were at physiological levels, i.e. the hEPCR down-regulating effect on hFVIIa generation still persisted in spite of hPC (119). Therefore, the EPCR-dependent regulation of FVIIa generation must exist on the unperturbed endothelium in vivo. EPCR thus seems to play an additional anticoagulant role in normal haemostasis by preventing FVIIa from being produced excessively under non-demand conditions (Fig. 1B).

Table 2: Reported dissociation constants (Kd) for the interaction between EPCR and their ligands in the human and murine settings. h, human; m, murine; sTF, soluble tissue factor; n.c., not calculated.

<table>
<thead>
<tr>
<th>EPCR</th>
<th>hPC/APC</th>
<th>hFVII/VIIa</th>
<th>hFX/Xa</th>
<th>mPC/APC</th>
<th>mFVII/VIIa</th>
<th>mFX/Xa</th>
</tr>
</thead>
<tbody>
<tr>
<td>hEPCR</td>
<td>30 nM</td>
<td>37 nM</td>
<td>&gt;1 μM</td>
<td>n.c.</td>
<td>292 nM</td>
<td>&gt;1 μM</td>
</tr>
<tr>
<td></td>
<td>32 nM</td>
<td>44 nM</td>
<td>3.5 μM</td>
<td>n.c.</td>
<td>397 nM</td>
<td>3.5 μM</td>
</tr>
<tr>
<td>mEPCR</td>
<td>58 nM</td>
<td>106 nM</td>
<td>n.c.</td>
<td>160 nM</td>
<td>106 nM</td>
<td>n.c.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>EPCR</th>
<th>hPC/APC</th>
<th>mFVII/VIIa</th>
<th>mFX/Xa</th>
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<tbody>
<tr>
<td>hEPCR</td>
<td>30 nM</td>
<td>150 nM</td>
<td>n.c.</td>
</tr>
<tr>
<td></td>
<td>32 nM</td>
<td>123 nM</td>
<td>n.c.</td>
</tr>
<tr>
<td>mEPCR</td>
<td>58 nM</td>
<td>397 nM</td>
<td>n.c.</td>
</tr>
</tbody>
</table>

The bold or bold-underlined residues in the murine sequence are conserved and the residues in italics changed with respect to the human sequence. The Gen Bank accession numbers of the sequences are CAG33229.1 and CAJ18383.1 for human and murine EPCR respectively. Numbering corresponds to human EPCR.
Nevertheless, FVIIa enzymatic activity is remarkably inefficient in the absence of TF. To visualise the effect of modulators under such conditions may therefore be difficult.

A different scenario emerges when TF is present i.e. outside the vessel lumen or in the injured vessel. The remarkably superior affinity of FVII for TF (~1-3 nM vs. ~40 nM for EPCR) (120) makes the interaction between EPCR and FVII negligible in the presence of TF. FVII uses its catalytic domain, Gla domain and, specially, its epidermal growth factor-1 (EGF-1) domain to bind to TF (121). FVII also uses its Gla domain to bind to negatively charged phospholipids or EPCR (109, 114, 115). When both receptors are on the cell surface, the avidity of TF for FVII would move the latter closer to the lipid bilayer and away from EPCR, whose interaction region with the Gla domain of FVII should be located on a higher plane, according to the predictions extracted from the solved crystal structure between EPCR and PC (108). There would remain the possibility that soluble EPCR (sEPCR), which circulates in plasma due to metalloprotease-induced shedding or (122, 123), to a lesser extent, to an alternative splicing of the EPCR gene (124, 125), could interfere with the interaction between FVIIa and TF. However, huge amounts of sEPCR, higher than those observed in physiological or pathological situations (126–128), are needed to impair the activation of hFX by the hFVIIa-TF complex on the cell surface (109).

The hEPCR-hFVIIa complexes have been shown to internalise into the cytoplasm of the endothelial cells in a similar fashion to the hEPCR-hPC/APC complexes (92). Thus, EPCR would also facilitate the transcytosis of FVIIa to clear it from the circulation and reach the extravascular space. Therefore, when FVIIa was pharmacologically administered, EPCR could contribute to creating extravascular reservoirs of FVIIa which could help to explain its long-lasting availability when exogenously administered. Also, once cleared out from the circulation, FVIIa would be in contact with numerous cells exhibiting TF on their membrane, VSMC or fibroblasts, for instance, which could contribute to the extravascular fibrin formation or to the triggering of cell signalling mechanisms. Nevertheless, due to the physiological circulating levels of FVII and PC, 10 and 60 nM, respectively, one could question the in vivo significance of these findings. However, when the circulating levels of FVII were studied in a large healthy population, the subjects who carried the Gly allele of the Ser219Gly substitution could account for the binding unability, a notion reinforced by the above mentioned high affinity observed between hFVIIa and mEPCR (Table 2) (111). However, somewhat unexpectedly, the occupancy of hEPCR by a catalytically inactive mutant of hFVII failed to provide a barrier protective response, unlike what happened with the catalytically inactive hAPC or zymogen hPC (54). Therefore, differences, as yet unknown, between the EPCR-PAR-1-dependent cell signalling mechanisms evoked by FVIIa or by PC/APC do exist.

Figure 3 summarises the effects known so far of the interaction between EPCR and FVIIa on the endothelial surface.

Since there are no more reports on the cell signalling effects of the FVIIa-EPCR complex, an appealing field of research is open for the near future. In this regard, caution must be taken with the experimental models used. Mice, the animals of choice in many of the studies of the APC-evoked cell signalling mechanisms, may not be the best option to approach the EPCR-dependent FVIIa-in vivo exerted actions. mFVII is a poor ligand for mEPCR as recently reported, the affinity being within the μM range (134). Furthermore, huge amounts of mFVIIa were unable to displace fluorescently labelled hAPC, which does interact with mEPCR, from the surface of murine endothelial cells (134). A key residue for EPCR binding in the Gla domain, i.e. Phe at position 4 which is present in hFVII, hPC and mPC, is replaced by Leu in mFVII (Table 1). This substitution could account for the binding inability, a notion reinforced by the above mentioned high affinity observed between hFVIIa and mEPCR (Table 2) (111). Also, these findings invite us to take the results of the APC-evoked cell signalling experiments performed in murine models cautiously, since, unlike what happens in the human setting, FVIIa will not interfere in the interaction between APC and EPCR.

Against these arguments, other authors have found a fair affinity between mFVIIa and mEPCR in the presence of TF (Table 2) (135). Soluble, not cell-based, TF (sTF) was used in these experiments. To date, there is no evidence of a biologically significant role of FVIIa complexed simultaneously to EPCR and TF on the cell surface in either themurine, or in the human, setting. Furthermore, the reported inability of soluble mEPCR (mEPCR) to alter the murine plasma prothrombin time, where clotting is driven by the mFVIIa-TF complex (134), argues against a relevant interaction between mEPCR and mFVIIa, even when TF is present.
Is FX/Xa a high affinity ligand of EPCR?

This interesting question has recently emerged and become an issue of controversy. Schuepbach et al. (136) have described how hFXa binds to human endothelial cells in a EPCR-dependent manner and that, as a result, PAR-1 is cleaved and cell signalling mechanisms are triggered. A co-receptor could be playing a role since a portion of hFXa remained bound to the endothelial cells still in the presence of large amounts of hAPC or a blocking anti-EPCR mAb. Although no hEPCR-hFXa affinity data were provided, the reported ability of hFXa to prevent the endothelial HPC activation, which was in the order of magnitude of hFVIIa, would suggest that the interaction could be relevant. (136). However, other authors have obtained the opposite results by using multiple experimental approaches: as assessed by flow cytometry, hFXa, still at a much higher molar excess, was unable to prevent the EPCR-dependent binding of the fluorescently labelled hAPC to human endothelial cells, unlike hPC, or hFVIIa (1, 134). Accordingly, 100-fold molar excess hFX could not compete with radiolabelled hFVIIa to bind to hEPCR-expressing cells. Consistently, when fluorescently-labelled hFXa was incubated with several hEPCR-expressing cells neither binding nor internalisation could be seen by confocal microscopy (157). Furthermore, the Kₜ of the interaction between hFX and hsEPCR obtained by SPR as well as the Kₛ of the binding of biotinylated hFXa to EPCR-expressing cells was in the μM range (134, 137) (➤Table 2). Therefore, the relevance of the interaction between hFX and hEPCR is questionable. Met instead of Glu domain Leu-8 is present in hFX (➤Table 1), and Leu-8 is directly involved in hEPCR binding (108). mPC also exhibits a Met instead of Leu-8 (➤Table 1). However, mPC does bind to mEPCR and, notably, hFX also binds to mEPCR with a relatively high affinity (134, 135) (➤Table 2). Finally, a cytoprotective variant of mPC was five-fold less efficient than its human counterpart in triggering cell signalling mechanisms in human endothelial cells (89). Collectively, these data suggest that Leu at position 8 in the Gla domain is mandatory for hEPCR binding while its substitution by Met would not preclude the interaction with mEPCR.

On the other hand, whether or not a co-receptor can improve the affinity between FX and EPCR in the human setting, as could be speculated after Schuepbach et al. (136), remains unanswered. Annexin 2 has been proposed to act as a receptor for FXa on the surface of endothelial cells in a Gla domain-dependent manner. Annexin 2 would permit FXa to activate PAR-1 in the absence of TF (138). Whether or not annexin 2 facilitates the interaction between FXa and EPCR has not been addressed to date. However, the above mentioned experiments by Puy et al and Sen et al were performed using endothelial cells, i.e. probably in the presence of the potential co-receptor (134, 137), and the results do not support a relevant interaction between EPCR and FXa on the endothelial surface, thus shedding doubt on such a hypothesis.

To complicate the picture even further, it must be also noted that, intriguingly, active site-inactivated hFXa has been shown to induce the dissociation of EPCR from caveolin-1 with the subsequent recruitment of PAR-1 to a protective pathway in human endothelial cells. Annexin 2 would not be involved since the Glu domain of hFXa was not required for this action, but the existence of an unknown endothelial FX/Xa receptor was suggested. These authors propose that FX/Xa would bind to such receptor rather than to EPCR, and the subsequent cross-talk between both receptors would trigger the reported cell signalling mechanisms (139).

The presence of TF could influence the association between FX/Xa and EPCR. Disse et al. have recently addressed this hypothesis and found that the presence of EPCR on the cell membrane was a requisite for FXa to exert PAR-1 and PAR-2-mediated signalling actions as taking part in the ternary complex hFXa-hFVIIa-TF in human TF/EPCR-expressing cell models. However, somewhat surprisingly FXa activation by the hFVIIa-TF complex was unaltered when EPCR was blocked (135). The same authors have reported data in the murine setting which point in the same direction (140). Again these data are at odds with the reported inability of active site-inhibited hAPC to induce an increase in the plasma levels of mFX when administered to mice in huge amounts to avoid endogenous mEPCR ligands being cleared from the circulation (137). These results are consistent with the inability of mEPCR to significantly delay the TF-dependent clotting time in murine plasma, which suggests a minor interaction between mEPCR and mFX or mFVII (134). mFX lacks Leu-8 which, moreover, is not substituted by Met but by Phe (➤Table 1), and no Kₛ calculations involving the mEPCR-mFX/Xa association have been performed to date.

In sum, the questions remain open. Why is there such a weak interaction between hFX and the soluble form of hEPCR, while the interaction seems to be relevant at the cell level? Is the presence of the ternary complex a sine qua non requirement for such an interaction? In this case, and since the FVIIa-TF complex should be properly aligned with FXa for optimal interaction (141), why is EPCR, which would move the Glu domain of FX away from the cell surface, unable to slow down its activation by the FVIIa-TF complex? Does the unidentified FX co-receptor plays a role? Further work is needed to address whether or not the interaction between EPCR and FX/Xa has to be considered biologically significant. The FXa-FVIIa-TF ternary complex has involved in physiological mechanisms like wound healing or tissue remodelling, but also in pathological processes such as exacerbated inflammatory response or metastasis progression (142–144). Does EPCR constitute a part of the inflammatory response? Or, on the contrary, would EPCR redirect signalling towards protective actions? The elucidation of these questions is of major importance.

Conclusions and perspectives

The cell signalling roles played by EPCR are a matter for active research. EPCR mediates the vast majority of the antiapoptotic, anti-inflammatory and barrier-protective effects attributed to APC, which engages PAR-1 for that purpose. FVII/IIa binds to EPCR with a similar affinity than PC/APC. In spite of the fact that FVII circulates at concentrations six times lower than PC, increasing evidence demonstrates that such an interaction results in physiologically relevant consequences. However, the interaction between FVII and EPCR becomes negligible when TF is present. There are
clues suggesting that FXa, when complexed with FVIIa and TF, interacts with EPCR. The receptor would help FXa to induce cell signalling events, which may change our current view of EPCR. However, contradictory results regarding this topic make further work necessary to clarify the nature of such an interaction. Finally, the species specificity of the binding between EPCR and its ligands invites us to be careful when choosing experimental models to address these questions.

Conflicts of interest
None declared.

References