Endothelial Cell Protein C Receptor
Role Beyond Endothelium?
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Endothelial cell protein C receptor (EPCR) is a type 1 transmembrane glycoprotein with homology to the major histocompatibility complex (MHC)-class I/CD1 family of molecules. EPCR was isolated and cloned as an endothelial cell-specific, high selectivity and high affinity binding protein for protein C (PC) and activated PC (APC). EPCR binds PC on the endothelial surface and presents it to the thrombin:thrombomodulin (TM) complex for activation (Figure 1). Thrombin bound to TM proteolytically activates PC and generates APC which exerts independent anticoagulant and cellular activities.2 The APC anticoagulant pathway is mediated by proteolytic inactivation of blood coagulation Factors Va and VIIIa with the contribution of several cofactors, including protein S, high density lipoproteins, phosphatidylserine, cardiolipin, and glucosylceramide to name a few. The APC cellular pathway on endothelium requires EPCR and the protease activated receptor-1 (PAR-1)3 which mediates APC’s cytoprotective effects, including alterations in gene expression profiles, antiapoptotic activity, antiinflammatory activity and protection of endothelial barriers4–10 (Figure 1). EPCR also regulates endogenous physiologic activation of PC by thrombin which is linked to PAR-1-dependent APC protective autocrine signaling in endothelium.11

EPCR signaling can decrease inflammation. APC binding to EPCR rescues baboons from E. coli sepsis.12 EPCR also has cardioprotective role in lipopolyscharide-induced endothoxemia in mice.14 In addition to the cell-surface EPCR, soluble EPCR lacking the transmembrane helix of native EPCR interacts with the integrin CD11b/CD18 (Mac-1) (αMβ2) (CR3) on leukocytes (Figure 1), suggesting that binding of soluble EPCR to Mac-1 might regulate leukocytes adhesion.15 Proteinase-3 (PR3), a serine protease with elastase-like properties stored in granules of neutrophils, binds both Mac-1 and soluble EPCR which may be implicated in APC mediated signaling and activation of PC on leukocytes, because soluble EPCR:PR3 complexes bind both APC and PC.15

Recent evidence indicates that EPCR is expressed on different cells beyond aortic endothelial cells. For example, EPCR is expressed on the surface of monocytes, CD56+ natural killer cells, neutrophils and eosinophils16–18, immature hematopoietic stem cells,19 brain capillary endothelial cells,13,20 EPCR is also expressed by embryonic giant trophoblast cells, and EPCR is critical for embryo development, because EPCR null mice die in mid-gestation.21 The study by Bretschneider et al, in the present issue of Circulation Research demonstrates functionally active EPCR in systemic vascular smooth muscle cells (SMCs).22 The data show that EPCR mediates APC signaling in SMCs, resulting in increases in intracellular [Ca2+], phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK-1/2) and DNA synthesis (Figure 1). Along with EPCR, PAR-1 is also necessary to transduce APC signaling in SMCs.

These findings are consistent with previous work demonstrating that both EPCR and PAR-1 are required for APC-mediated activation of ERK1/2 and [Ca2+], signaling in systemic and cerebral capillary endothelial cells.5,20 These findings open a new chapter in biology of EPCR by demonstrating that EPCR can be expressed in cells which have different biological functions from endothelial and hematopoietic cells. In this context, it is of note that the microarray gene expression profiling of human cerebral SMCs and astrocytes have indicated the presence of EPCR mRNA and showed that the levels of EPCR transcripts in these brain cells were not altered by normal aging in humans or aging Alzheimer’s type (R. Zidovetski, N. Chow, B. Zlokovic, unpublished data, 2007). The exact role of EPCR and how its expression is regulated in nonendothelial cell types including systemic SMCs, primitive hematopoietic stem cells or cerebral SMCs, remain to be elucidated.

A regulatory element 5.5 kb upstream to the human EPCR gene (hEPCR) translation start site is essential for driving hEPCR expression in endothelium and primitive hematopoietic cells which share a common precursor.23 It would be interesting to find out whether the same site in hEPCR is responsible for driving hEPCR expression in human SMCs.22 The present findings by Bretschneider et al22 raise not only a possibility that EPCR could play an important role in regulating the biology of vascular SMCs, but also suggest that its expression in SMCs might have implications for the pathogenesis of cardiovascular diseases such as atherosclerosis, and by extension, cerebral amyloid angiopathy in Alzheimer’s disease. EPCR may be a potential therapeutic target: novel EPCR agonists might modify cytoprotective APC/PC cellular pathways in injured or inflamed blood vessels.

We still have much to learn about the role of EPCR beyond the endothelium, and perhaps in the endothelium itself. For
example, Dr Esmon has suggested that EPCR appears to be able to translocate from the plasma membrane into the nucleus, a cellular trafficking pattern that is not observed with its Cys to Ser mutant.24 One may speculate that EPCR may internalize its endogenous ligands PC and APC into the cytoplasm and into the nucleus, which in turn could have important implications for regulation of gene expression and control of APC activity. Figure 2a shows dense punctate staining of APC on the surface of brain endothelial normoxic cells, which is completely removed by pretreatment of cells with an antibody that blocks APC binding site on EPCR (Figure 2b),25 demonstrating that APC binding to the cell is highly EPCR-dependent, as suggested.1 On the other hand, Figure 2c shows that APC can be transported intracellularly and into the nucleus of brain endothelial cells under hypoxic conditions (Figure 2c), which is associated with cell protection and survival, as reported.13 This transport pattern, however, completely disappears if APC binding to EPCR is blocked (Figure 2d), and cells die as a result of APC unavailability, as shown by faint APC staining on cell debris. Thus, the role of EPCR-APC-PAR-1-dependent intracellular cytoprotective signaling and of EPCR-mediated intracellular trafficking and internalization of APC/PC in normal and injured vascular endothelial cells and SMCs, may open a new era in our understanding of EPCR biology, its physiological and pathological functions, and therapeutic opportunities for

![Diagram](image-url)

**Figure 1.** EPCR binds protein C (PC) on endothelium and presents it to TM (thrombomodulin)-thrombin complex for activation by thrombin. Activated PC (APC) dissociates from EPCR and TM-thrombin complexes and inactivates coagulation factors Va and Vila into inactive forms V and VIIa, promoting anticoagulation. APC bond to EPCR activates the protease activated receptor-1 (PAR-1) which binds the Gq and Gi proteins to activate phospholipase(s) (PLC) in SMCs and endothelium resulting in IP3 (inositol triphosphate)-mediated release of intracellular Ca\(^{2+}\), activation of mitogen-activated kinases ERK1/2 and stimulation of DNA synthesis.22 In injured endothelium, EPCR-APC-mediated PAR-1 activation blocks mitochondria-mediated apoptotic pathway (eg, inhibition of p53 and Bax, activation of antiapoptotic Bcl-2 gene, blockade of caspase-9), and death receptor-mediated pathway through inhibition of caspase-8. Whether the same antiapoptotic signaling exists in injured SMCs is unknown. Soluble form of EPCR (sEPCR) binds APC/PC and forms complexes with proteinase-3 (PR3) which binds to Mac-1 on leukocytes and activates antiinflammatory signaling. EPCR-APC–dependent PAR-1 activation in leukocytes and endothelium blocks the expression of proinflammatory genes by preventing nuclear translocation of nuclear factor-kB (NF-kB). EPCR translocates itself from the plasma membrane to the nucleus and may internalize APC into injured vascular cells, but the physiological importance of EPCR internalization alone and/or of its ligands remains at present elusive.

![Images](image-url)

**Figure 2.** Human brain endothelial cells were cultured under normoxic and hypoxic (< 1% oxygen, no glucose) conditions, as we described.10,13,20 Human plasma-derived APC (20 nM) was incubated with cells for 4 hour under either normoxic (A and B) or hypoxic (C and D) conditions in the absence (A and C) and presence (B and D) of an anti-EPCR antibody (RCR-252) which blocks APC binding site on EPCR.25 APC binding was detected by an APC/PC-specific antibody (C3), as described,11,13 using confocal microscopy. In normoxic cells, a punctate staining shows binding of APC to the plasma membrane (A) and complete displacement of APC binding by anti-EPCR antibody (B). In hypoxic cells, APC is internalized into the cytoplasm and nucleus which is associated with the cell survival (C). In contrast, anti-EPCR antibody prevents APC binding to hypoxic cells which is associated with loss of protection and cell death (D), as reported.13
disorders of the cardiovascular system and cerebral circulation.

References


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