EPCR-Dependent Activation of Protein C is Stimulated by Platelet Factor 4

Rachel L. Ochoa, Laurent O. Mosnier, John H. Griffin, The Scripps Research Institute

Protein C (PC) is a serine protease zymogen in blood that provides a major anticoagulant pathway of blood coagulation. Activated protein C (APC) has both anticoagulant and cytoprotective activity. PC is activated by thrombin in a reaction catalyzed by two receptors on the endothelial surface, thrombomodulin and the endothelial protein C receptor (EPCR). Platelet factor 4 (PF4), a cationic platelet α-granule protein released during platelet activation, was shown to enhance the activation of PC by the thrombin-thrombomodulin complex. Since both EPCR and PF4 interact with the amino-terminal, γ-carboxylglutamic acid domain of PC, our objective was to determine whether the enhancements of PC activation by PF4 and EPCR are mutually exclusive or independently additive. To answer this question, endothelial cells (EA.hy926) expressing thrombomodulin and EPCR were used to study the binding and activation of PC. APC was bound to EPCR on cells in the presence and absence of PF4 with similar affinities (99 nM and 132 nM, respectively), implying no significant competition between PF4 and EPCR for binding of APC. Normal PC activation on endothelial cells was dependent on EPCR because an anti-EPCR antibody increased the K_M for PC activation approximately 30-fold. PF4 increased the activation of PC in the presence of EPCR but not when PC binding to EPCR was blocked by an anti-EPCR antibody. This indicates stimulation of PC activation by PF4 on endothelial cells is EPCR-dependent. These results combined with previous literature data suggest that EPCR and PF4 have independent binding sites on the Gla domain of PC. Therefore, PF4 enhances PC activation on endothelial cells without interfering with EPCR- dependent activation of PC.