Endothelial cells play a pivotal role in the regulation of vascular function. Not only do endothelial cells modulate vascular tone and control the initiation and progression of vascular inflammation through regulated secretion of vasoactive agents and by surface expression of adhesion molecules, but they also provide a hemocompatible vessel lining by molecular control of platelet aggregation, coagulation, and fibrinolysis. One of the many vasoactive agents important in maintaining both vascular integrity and hemostasis is thrombin, the main effector serine protease of the coagulation cascade. Physiologically, thrombin is short lived in the circulation, and in the context of a normal endothelium, thrombin activates the protein C system to terminate its own production. However, in pathological conditions, at sites of vascular injury, thrombin has a host of direct actions. It is a potent activator of platelets, it induces shape and permeability changes of endothelial cells, it mobilizes adhesion molecules to the endothelial surface, it stimulates autacoid and proinflammatory cytokine production, it regulates blood vessel diameter by modulating endothelium-dependent vasodilation and endothelium-independent vascular smooth muscle vasconstriction, and it stimulates vascular cell growth. Endothelial effects of thrombin have been attributed to NO production through activation of endothelial NO synthase (eNOS), which requires phosphorylation of Ser1179. Exact mechanisms whereby thrombin regulates eNOS phosphorylation remain unclear, although Ca$^{2+}$/calmodulin and Akt have been implicated to be critical.

In the current issue of *Hypertension*, Motley et al delineate some molecular processes by which thrombin influences eNOS in human endothelial cells. Using a multidisciplinary pharmacological and molecular approach, they demonstrate that Ser1179 phosphorylation and activation of eNOS by thrombin occurs through a Ca$^{2+}$-dependent, protein kinase C (PKC)δ-sensitive, but phosphatidylinositol 3-kinase (PI3K)/Akt-independent pathway (Figure).

What is intriguing about these findings is that, unlike other vasoactive agonists, which regulate eNOS through well-defined PI3K/Akt-sensitive pathways, Akt activation does not seem to be obligatory for eNOS regulation by thrombin, despite the fact that thrombin stimulates PI3K/Akt signaling and that Akt can directly serine phosphorylate NO synthase. Others have also shown that thrombin induces eNOS phosphorylation independently of Akt, but rather through a 5′-AMP–activated protein kinase–sensitive pathway.

The question that arises is why is Akt not indispensable for eNOS activation by thrombin when it is so critical for other agonists? It may be possible that functional responses mediated by thrombin through Akt signaling in endothelial cells are indeed NO independent. In support of this, Viswambharan et al demonstrated that thrombin-induced endothelial tissue factor expression involves activation of PI3K but not of Akt/eNOS. Other thrombin-mediated endothelial effects, which may not necessarily involve NO, include synthesis and secretion of platelet-activating factor, von Willebrand factor, tissue plasminogen activator, and type 1 plasminogen activator inhibitor.

Another consideration is that the observations of Motley et al should be interpreted within the context of the experimental paradigm. From a kinetic viewpoint, whereas thrombin induced rapid Ca$^{2+}$-dependent serine phosphorylation of eNOS, that is, within 3 minutes, Akt phosphorylation was delayed, and maximal responses were achieved only once eNOS activation returned to the basal state, that is, within 20 minutes. However, what we do not know is what happens to eNOS activation and NO production in response to thrombin in the long term. It may be possible that prolonged release of NO in stimulated cells is independent of a detectable rise in intracellular Ca$^{2+}$ but may be because of activated Akt/PI3K. Such findings have been shown previously in endothelial cells.

The new paradigm that is suggested is provocative and warrants further deliberation. First, it would have been interesting to know whether thrombin-induced activation of eNOS through Ca$^{2+}$ and PKCδ-dependent pathways are functionally linked to endothelial cell changes in the context of Akt inhibition or downregulation. Second, it is unclear exactly what the functional significance of PKCδ is relative to other PKC isoforms. In fact, PKCδ may be more important in maintaining endothelial barrier function and controlling inflammatory responses than in regulating NO-dependent vascular tone. Recent studies have demonstrated that thrombin-mediated vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in endothelial cells involves the coordinate activity of PKCδ/nuclear factor κB and PKCγ. Third, differential endothelial cell signaling, Ca$^{2+}$/PKC/eNOS on the 1 hand and PI3K/Akt on the other hand, may be mediated by thrombin through different G protein–coupled protease-activated receptors (PARs). To date, 4 PARs have been identified, all of which are expressed, to varying degrees, in the endothelium. Only PAR1 and PAR3 are directly activated by thrombin in the endothelium. Motley et al did not explore the receptor subtype through which thrombin influences eNOS and Akt, but it may be
Thrombin signaling through G protein–coupled PAR in endothelial cells. Activation of eNOS by thrombin involves multiple pathways, including Ca\(^{2+}\)/calmodulin, PKC\(\beta\), and PI3K/Akt. Thrombin-induced activation of Akt may also signal through eNOS/NO-independent pathways and may negatively regulate PKC\(\beta\). eNOS-derived NO, in turn, leads to activation of downstream signaling cascades in vascular smooth muscle cells (VSMCs), which regulate vascular tone, growth, and inflammation. Dashed line indicates possible inhibitory effect (Reference 19).

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### References

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