

# Protease-activated receptors differentially regulate endothelial nitric oxide synthase

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Protease-activated receptors (PARs) are a family of G-protein-coupled receptors that are primarily expressed in cells of the vasculature and known for their involvement in regulation of vascular tone. These receptors induce endothelium-dependent relaxation via production and release of a potent vasodilator, nitric oxide (NO), and therefore, play an important role in the function of the endothelium. Disruption of endothelial function can lead to the development of cardiovascular diseases such as atherosclerosis and hypertension. There are four members of the PAR family, PAR-1 through -4, and unlike traditional receptors, which are activated by coupling with a ligand, these receptors are uniquely irreversibly activated via cleavage by serine proteases that expose a neo-N-terminus, which acts as a self-activating peptide. PAR-1, -3 and -4, are directly activated by thrombin, a serine protease responsible for platelet aggregation, endothelial cell activation, and other important responses in the vasculature. PAR-2 is activated by trypsin-like enzymes and can respond to thrombin only through transactivation by PAR-1.

It was known that several members of the PAR family modulate the production of NO via post-translational modification of endothelial nitric oxide synthase (eNOS). PARs have been shown to phosphorylate this enzyme at two specific regulatory sites. When eNOS-Ser-1177/-1179 (human/bovine) is phosphorylated, there is an increase in eNOS activity followed by an immediate increase in the production of NO. In contrast, when eNOS-Thr-495/-497 (human/bovine) is phosphorylated, eNOS activity is down regulated, which leads to a

decrease in NO production. Motley and colleagues have previously demonstrated the reciprocal regulation of the phosphorylation of these two sites on eNOS by PARs using bovine aortic endothelial cells (BAECs) and human umbilical vein endothelial cells (HUVECs). In addition, PARs phosphorylate the two eNOS sites via different G protein-dependent signal transduction pathways. They showed that thrombin and a selective PAR-1 activating peptide (AP), TFLLR, modulate eNOS negatively by phosphorylating eNOS at the Thr-495 site and signals through a Rho/ROCK dependent-pathway that is coupled to G12/13. Activation of PAR-2 via PAR-1 transactivation or using an activating peptide specific for PAR-2, SLIGRL, phosphorylates eNOS at Ser1177 through Gq, which is coupled to Ca<sup>2+</sup>, a PKC- $\delta$ -sensitive, and PI3K/AKT-independent signaling pathway.

In the March 2016 issue of *Experimental Biology and Medicine* Tillery et al provide evidence of a deviation from the classical role of PARs in mediating the regulation of eNOS phosphorylation in humans. In this study, primary human coronary artery [endothelial cells](#) (HCAECs) were used as a model system to characterize the signaling potential of PARs. Thrombin was observed to primarily regulate the positive site of eNOS, inducing phosphorylation of eNOS-Ser-1177. Activation of PAR-1 and PAR-2 using specific activating peptide ligands phosphorylated both regulatory sites with evidence of dual signaling pathways. However, only PAR-1 activation resulted in an increase in NO metabolites, which favors vasodilation, while selective PAR-2 activation prevented NO production, which favors vasoconstriction. In addition, they were able to characterize the functional role of PAR-3, which induced eNOS-Thr-495 phosphorylation only; whereas PAR-4, which was not expressed in these cells. These are novel findings, which characterize PAR-mediated eNOS/NO signaling in relevant HACECs in the critical vasculature altered in ischemic heart disease. Tillery reflected, "Being able to understand PAR signaling in the human vasculature will aid in targeting specific molecular sites in the vasculature for therapeutic

treatments to circumvent endothelial dysfunction and decrease the prevalence of other associated cardiovascular diseases."

Dr. Steven R. Goodman, Editor-in-Chief of *Experimental Biology and Medicine*, said "the studies by Motley and colleagues provides unique insight into PAR signaling which could lead to new therapies to treat vascular diseases".

**More information:** L. C. Tillery et al. Featured Article: Differential regulation of endothelial nitric oxide synthase phosphorylation by protease-activated receptors in adult human endothelial cells, *Experimental Biology and Medicine* (2016). [DOI: 10.1177/1535370215622584](https://doi.org/10.1177/1535370215622584)

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