ENDOTHELIAL NOS, SPHINGOSINE-1-PHOSPHATE IN CARDIOVASCULAR AND INFLAMMATORY PROCESSES

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Endothelial nitric oxide (eNOS) has evolved to be tightly controlled by co- and post-translational lipid modifications, phosphorylation on multiple residues and regulated protein-protein interactions. In general, in the basal state, eNOS produces a constant low amount of NO; when stimulated by a variety of stimuli eNOS activity is enhanced and NO concentration increases. eNOS is a Ca^{2+}/calmodulin (CaM) dependent enzyme localized to the caveolae where it associates with caveolin-1, that maintains the enzyme in an inactive state. Ca^{2+}/CaM weakens the eNOS-caveolin-1 interaction and thus the enzyme is activated by combined loss of caveolin-1 interaction and the direct effect of Ca^{2+}/CaM. Recent studies have expanded the regulation of eNOS to sphingolipid signalling. In particular, sphingosine-1-phosphate (S1P) has emerged as a potent and versatile bioactive molecule. S1P is formed by removal of the amide-linked fatty acid side chain of ceramide through the action of the enzyme ceramidase. Production of S1P then occurs by phosphorylation of sphingosine at the 1-OH position by the enzyme sphingosine kinase (SPK). S1P can act as an extra-cellular ligand, activating specific G-protein-coupled sphingolipid receptors in the plasma membrane. Alternatively, S1P produced following activation of various plasma membrane receptors can fulfil the role of a second messenger and stimulate Ca^{2+} channel on the endoplamic reticulum. In particular we investigated on the intracellular second messenger role of S1P in eNOS activation in aortic vessel and on the contribution of this alternate pathway in the control of vascular tone. S1P causes an endothelium-dependent vasorelaxation in rat aorta which is PTX sensitive, inhibited by L-NAME and mainly dependent on hsp90. When rat aorta rings were incubated with the SPK inhibitor $_{DL}$-threo-dihydrosphingosine (DTD) there was a concentration dependent reduction of Ach-induced vasorelaxation implying a consistent contribution of sphingolipid pathway in Ach-induced vasorelaxation, through sphingosine release and phosphorylation. Co-immunoprecipitation consistently showed an increased association of hsp90 with eNOS following exposure to S1P as well to BK or calcium ionophore A-23187. Interestingly, as opposite to A-23187, BK and S1P effect were significantly inhibited by pre-treatment with the SPK inhibitor DTD. Our data demonstrate that an interplay exists among eNOS, hsp90 and intracellularly generated S1P where eNOS coupling to hsp90 is a major determinant for NO release.