Abstract: P552

Role of PI3K/Akt and MEK/ERK signalling in Epac-mediated endothelial barrier stabilisation and survival

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Topic(s):
Vascular Tone, Permeability, Microcirculation

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S135

Background and Aims: Activation of the cAMP/Epac signalling stabilises endothelial barrier function. cAMP/Epac also activates PI3K/Akt and MEK/ERK signalling in diverse cell types, but the impact of this activation on endothelial barrier function is largely unknown. Here the role of PI3K/Akt and MEK/ERK signalling in cAMP/Epac-mediated endothelial barrier stabilisation was analysed.

Methods: Endothelial barrier function was analysed in cultured human umbilical vein endothelial cells (HUVECs) by measuring albumin flux. A modified cAMP analogue 8-pCPT-2'-O-Me-cAMP was used to specifically activate cAMP/Epac signalling. Activation of PI3K, Akt, MEK, and ERK was measured by Western blotting. Caspase activity was determined by Caspase-Glo kit. qPCR was used to quantify the expression level of PI3K isoforms. Cytoskeletal proteins and cell-cell junctions were visualised by immunohistochemistry using phalloidin TRITC and antibody against VE-cadherin, respectively. Endothelial cell proliferation was determined by expression of Ki67.

Results: The Epac agonist reduced basal and attenuated thrombin-induced endothelial hyperpermeability; this was accompanied by an activation of PI3K/Akt and MEK/ERK signalling. qPCR demonstrated that HUVECs express PI3Ka, PI3Kβ, and PI3Kγ but not PI3Kδ isoforms. Western blotting confirmed that the Epac agonist preferentially activates PI3Ka and PI3Kβ isoforms. Inhibition of the MEK/ERK using U0126 but not the PI3K/Akt pathway by Akt inhibitor VIII, potentiated the endothelial barrier-protective effects of cAMP/Epac signalling. Inhibition of MEK/ERK signalling in the presence of the Epac agonist induced reorganisation of the actin cytoskeleton to the cell periphery, reduced stress fibre formation, and enhanced VE-cadherin localisation at cell-cell junctions. Moreover, the Epac agonist promoted endothelial cell (EC) survival but not proliferation via reduction in activities of pro-apoptotic caspases in a PI3K/Akt and MEK/ERK signalling-dependent manner as determined by respective selective pharmacological inhibitors.

Conclusion: Our data demonstrate that stimulation of the cAMP/Epac axis simultaneously activates PI3K/Akt and MEK/ERK pathways, which govern the pro-survival effects of Epac signalling on ECs. Inhibition of MEK/ERK but not PI3K/Akt signalling enhances barrier-stabilising and barrier-protective effects of cAMP/Epac activation.