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Stimulation of the EP_3 receptor causes lung oedema by activation of TRPC6 in pulmonary endothelial cells

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Shareable abstract (@ERSpublications)
EP_3 activation triggers pulmonary oedema *via* G_i-dependent activation of PLC and subsequent tyrosine phosphorylation of TRPC6. In PAF-induced lung oedema this TRPC6 activation coincides with ASMase-dependent caveolar recruitment of TRPC6. https://bit.ly/34P3d13

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Abstract

Background Prostaglandin E_2 (PGE_2) increases pulmonary vascular permeability by activation of the PGE_2 receptor 3 (EP_3), which may explain adverse pulmonary effects of the EP_1/EP_3 receptor agonist sulprostone in patients. In addition, PGE_2 contributes to pulmonary oedema in response to platelet-activating factor (PAF). PAF increases endothelial permeability by recruiting the cation channel transient receptor potential canonical 6 (TRPC6) to endothelial caveolae *via* acid sphingomyelinase (ASMase). Yet, the roles of PGE_2 and EP_3 in this pathway are unknown. We hypothesised that EP_3 receptor activation may increase pulmonary vascular permeability by activation of TRPC6, and thus, synergise with ASMase-mediated TRPC6 recruitment in PAF-induced lung oedema.

Methods In isolated lungs, we measured increases in endothelial calcium (ΔCa^{2+}) or lung weight (Δweight), and endothelial caveolar TRPC6 abundance as well as phosphorylation.

Results PAF-induced ΔCa^{2+} and Δweight were attenuated in EP_3-deficient mice. Sulprostone replicated PAF-induced ΔCa^{2+} and Δweight which were blocked by pharmacological/genetic inhibition of TRPC6, ASMase or Src-family kinases (SrcFK). PAF, but not sulprostone, increased TRPC6 abundance in endothelial caveolae. Immunoprecipitation revealed PAF- and sulprostone-induced tyrosine-phosphorylation of TRPC6 that was prevented by inhibition of phospholipase C (PLC) or SrcFK. PLC inhibition also blocked sulprostone-induced ΔCa^{2+} and Δweight, as did inhibition of SrcFK or inhibitory G-protein (G_i) signalling.

Conclusions EP_3 activation triggers pulmonary oedema *via* G_i-dependent activation of PLC and subsequent SrcFK-dependent tyrosine phosphorylation of TRPC6. In PAF-induced lung oedema, this TRPC6 activation coincides with ASMase-dependent caveolar recruitment of TRPC6, resulting in rapid endothelial Ca^{2+} influx and barrier failure.