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Obligatory role for PKCδ in activation of store-operated TRPC1 channels in vascular smooth muscle

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Introduction: Stimulation of store-operated channels (SOCs) composed of TRPC1 in vascular smooth muscle cells (VSMCs) regulate cell contraction, proliferation and migration associated with vascular disease (1,2). Therefore it is important to understand how TRPC1 SOCs are activated. Stimulation of TRPC1 SOCs and PKC phosphorylation by store depleting agents that do not increase intracellular Ca^{2+} concentration (e.g. BAPTA, TPEN) require PLCβ1 activity. This indicates that the PKC isoform involved requires diacylglycerol but is Ca^{2+}-sensitive - characteristics of the novel group of PKC isoforms (e.g.δ, ε, η, θ). Since PKCδ has been linked to activation of SOCs in airway smooth muscle and is linked to regulating vascular tone (3), we focused on this isoform in activating TRPC1 SOCs.

Method: Freshly isolated mouse mesenteric artery VSMCs were used for whole-cell and single channel electrophysiological recordings and immunocytochemistry studies in the presence of store depleting agents as previously described (1,2). Isolated mouse mesenteric arteries were used for isometric tension recording to study the effects of the specific PKCδ inhibitor δV1-1-Tat on pre-contracted arteries.

Results: The selective PKCδ inhibitor peptides, δV1-1-TAT and δPKC, inhibited whole-cell and single channel TRPC1 currents in freshly isolated mouse mesenteric artery VSMCs by over 80%. Pre-treatment of mesenteric artery segments or single VSMCs with store depleting agents induced associations between TRPC1 and PKCδ at the plasma membrane using co-immunoprecipitation, immunocytochemical staining, and proximity ligation assay. Store depleting agents evoked relaxation of pre-contracted mesenteric arteries which were inhibited by δV1-1-Tat. Interestingly, in the presence of the large conductance Ca^{2+}-activated K^+ channel blocker iberatoxin, store depletion evoked vasoconstriction which was reduced by δV1-1-Tat.

Conclusions: These results indicate that PKCδ is likely to be the dominant PKC isoform involved in activating TRPC1 SOCs, and mediates store-operated changes in vascular reactivity.

References:

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2. Shi *et al* (2017) J Physiol **595**, 1039-1058 3. Garcia *et al* (2011) Channels **5**, 210-214