TRPC5 channel-Caveolin-1-eNOS signalplexes coordinate interplay between Ca^{2+} and NO signals in endothelial cells

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The cell signaling molecules nitric oxide (NO) and Ca^{2+} regulate diverse biological processes through closely coordinated activities. However, signaling protein complexes referred to as "signalplexes" that underlie the interplay of Ca^{2+} and NO pathways remain unclear in many tissues and cell types including vascular endothelial cells. Here we demonstrate the physical and functional interaction of the receptor-activated Ca^{2+} -permeable TRPC5 channel with Ca^{2+} -dependent endothelial NO synthase (eNOS) in endothelial cells. Upon stimulation of a G-protein-coupled ATP receptor, Ca^{2+} influx via receptor-activated TRPC5 channels elicits NO production by eNOS, which in turn induces secondary activation of TRPC5 channels via cysteine *S*-nitrosylation. TRPC5 is co-immunoprecipitated with eNOS and the scaffolding protein caveolin-1. Mutations in the caveolin-1-binding domains of both TRPC5 and eNOS impair their association and also disrupt Ca^{2+} influx and NO production, suggesting that caveolin-1 is the scaffold around which TRPC5 and eNOS assemble into the signalplex. Interestingly, intracellular Ca^{2+} elevation dissociates eNOS from caveolin-1 but enhances the association between TRPC5 and eNOS at the plasma membrane. This relieves eNOS from negative regulation by caveolin-1 and may enhance the production of NO in the vicinity of TRPC5, leading to an efficient secondary activation of TRPC5 via *S*-nitrosylation. Thus, our study provides evidence that TRPC5 channel-caveolin-1-eNOS signalplexes are critical in coordinating NO and Ca^{2+} signaling in vascular endothelial cells.