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Rapid flow-induced $G\alpha_{q/11}$ activation occurs upstream of Piezo1 activation

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Endothelial mechanotransduction is the process by which mechanical stimuli are sensed by endothelial cells (ECs) and transduced into biochemical signals and ultimately into physiological responses. Identifying the primary mechanosensor(s) and describing the mechanism(s) by which they receive and transmit the signals has remained a central focus within the field. The heterotrimeric G protein, $G\alpha_{q/11}$, is proposed to be part of a macromolecular complex together with PECAM-1 at the cell-cell junction of ECs and may serve as a primary mechanosensor as it is rapidly activated within seconds of flow onset. The mechanically-activated cation channel, Piezo1, has recently been implicated due in part to its involvement in mediating early responses, such as calcium influx and ATP release. In this study, we used *in situ* proximity ligation assay (PLA) to investigate the role of Piezo1 in the rapid shear stress-induced activation of $G\alpha_{q/11}$. We show that the flow-induced dissociation of $G\alpha_{q/11}$ from PECAM-1 in ECs at 15 sec is abrogated by BIM-46187, a specific inhibitor of $G\alpha_{q/11}$ activation, suggesting that $G\alpha_{q/11}$ activation is a prerequisite for PECAM-1/$G\alpha_{q/11}$ dissociation. Although siRNA knockdown of Piezo1 caused a dramatic decrease in PECAM-1/$G\alpha_{q/11}$ association in the basal condition, it had no effect on their flow-induced dissociation. Interestingly, siRNA knockdown of Piezo1 caused a marked decrease in PECAM-1 expression. Selective blockade of Piezo1 with ion channel inhibitors also had no effect on flow-induced PECAM-1/$G\alpha_{q/11}$ dissociations despite having an inhibitory effect on Akt phosphorylation. Rapid flow (15 sec) also led to increased association of $G\beta_1$ with Piezo1 as well as with the p101 subunit of phosphoinositide 3-kinase (PI3K), which were both blocked in the presence of the $G\beta\gamma$ inhibitor, gallein. Taken together, our results indicate that flow-induced activation of Piezo1 is downstream of G protein activation.

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