Shear stress-induced NO production is dependent on ATP autocrine signaling and capacitative calcium entry

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Introduction: Flow-induced production of nitric oxide (NO) by endothelial cells plays a fundamental role in vascular homeostasis. However, the mechanisms by which shear stress activates NO production remain unclear due in part to limitations in measuring NO, especially under flow conditions. Shear stress elicits the release of ATP, but the relative contribution of autocrine stimulation by ATP to flow-induced NO production has not been established. Furthermore, the importance of calcium in shear stress-induced NO production remains controversial, and in particular the role of capacitive calcium entry (CCE) has yet to be determined.

Materials and Methods: We have utilized our unique NO measurement device to investigate the role of ATP autocrine signaling and CCE in shear stress-induced NO production. Our device consists of a parallel plate flow chamber in which a NO sensitive electrode is located in a stagnant upper compartment separated from the ECs and the flow field by a porous membrane

Results and Discussion: We found that endogenously released ATP and downstream activation of purinergic receptors and CCE plays a significant role in shear stress-induced NO production. ATP-induced eNOS phophorylation under static conditions is also dependent on CCE. Inhibition of protein kinase C significantly inhibited eNOS phosphorylation and the calcium response. To our knowledge, we are the first to report on the role of CCE in the mechanism of acute shear stress-induced NO response. In addition, our work highlights the importance of ATP autocrine signaling in shear stress-induced NO production.

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