Neutrophil-induced regulation of [Ca2+]i in human endothelial cell: role of redox sensitive ion channels
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Abstract: This study evaluates the changes in the intracellular Ca2+ concentration [Ca2+]i, in response to polymorphonuclear neutrophil (PMN) and the roles of redox sensitive ion channels in modulating the [Ca2+]i, in human endothelial cell (EC). Human pulmonary artery endothelial cells (HPAEC) maintained as monolayers in culture were loaded with fluorescent Ca2+ indicator Fura-2. Adding of freshly isolated PMN (stimulated by 1µM fMLP just before adding) caused a rapid increase in [Ca2+]i in endothelial cells. Prior stimulation with thrombin or thapsigargin to deplete the ER Ca2+ store partly inhibited the PMN induced increase in [Ca2+]i, and blocking of the IP3 receptor and plasma membrane TRPC channels by 2-APB significantly inhibited the PMN induced increase in [Ca2+]i. In the absence of extracellular Ca2+, PMN increased the EC [Ca2+]i, suggesting the release of Ca2+ from ER store and subsequent addition of 2mM Ca2+ caused the slow increase in [Ca2+]i, indicating the entry of Ca2+ through the channels in the plasma membrane yet to be identified. Pretreatments with antioxidant agents, i.e superoxide dismutase (SOD), NADPH oxidase inhibitor DPI, and PEG-catalase attenuated the PMN induced increases in [Ca2+]i in EC. These results suggest that signaling events during activated neutrophil contact with endothelial cell causing the increase in [Ca2+]i is mediated by redox sensitive mechanism, that leads to increase in [Ca2+]i partly due to release of Ca2+ from ER Ca2+ store and also entry of extra cellular Ca2+ through redox sensitive TRP channels on the plasma membrane of endothelial cells.
Calcium oscillations induced by ATP in human umbilical cord smooth muscle cells
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Oscillations in smooth muscle calcium levels have been described, but the origin and function of these has not yet been fully determined. In cultured human umbilical cord smooth muscle cells (HUCSM), we found that ATP, at a micromolar dose that activates P2Y receptors, induced a steady calcium oscillation in previously quiescent cells and the calcium entry pathway was shown to be via TRP channel activation. These oscillations were quenched by higher, millimolar doses of ATP or membrane depolarization by high, bath K⁺. In addition, when external calcium was removed by EGTA, micromolar ATP could not induce oscillations. Surprisingly, if internal calcium stores were not pre depleted, we found that only a small proportion of the calcium increase following P2Y receptor activation, could be blocked by the P2 antagonist suramin, or the TRP channel inhibitor, 2APB, suggesting an alternative calcium entry pathway was activated under these conditions. However, if internal stores were pre depleted by EGTA or high K⁺, PLC or G-protein antagonists could almost fully reverse the calcium increase induced by ATP. After 60 hours incubation at 1-2% O₂, the calcium oscillation frequency and amplitude were increased. Taken together, these data suggest that external calcium entry maintains the calcium oscillation induced by ATP but the status of the internal calcium store directs the precise calcium entry pathway utilised and that chronic hypoxia can modulate ion channel activity in these cells.

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