HEMICHANNEL INVOLVEMENT IN CA2+ DYNAMICS AND CONTRACTILITY OF SMOOTH MUSCLE CELLS IN ACUTELY ISOLATED SMALL MESENTERIC ARTERIES

Bol[1] M., De Bock[1] M., De Vuyst[1] E., Wang[1] N., Decrock[1] E., Monsalvo[1] J., Decaluwe[2] K., Vanheel[1] B., Van de Voorde[2] J., Leybaert[1] L.

Intracellular Ca^{2+} mediates a variety of vascular endothelial and smooth muscle cell functions. Smooth muscle cells (SMC) respond to biological activators with oscillatory and propagating rises in $[Ca^{2+}]_i$ that are highly organized in both time and space. Gap junctions (GJs) play a crucial role in the communication between vascular cells and in the synchronization of Ca^{2+} signals thereby tightly controlling the level of vasoconstriction. Before being incorporated into GJs, connexin (Cx) hemichannels reside in the plasma membrane in a closed state. Recent evidence suggests that hemichannels can be opened by various messengers and conditions, thereby forming a pore that allows the passage of ATP and ions. Using confocal microscopy and the Ca^{2+} sensitive dye Fluo-3, we examined the role of hemichannels in dynamic Ca^{2+} responses of SMC in intact acutely isolated small rat mesenteric arteries. Furthermore, we assessed the involvement of these signalling partners in contractile responses of small mesenteric arteries using a wire myograph for isometric tension measurements. Importantly, the experimental conditions were such that vasomotion, characterized by synchronized Ca^{2+} signals, was avoided because in that case gap junctions between SMC and myo-endothelial gap junctions are expected to contribute. Norepinephrine (NOR, 3 μM) induced Ca^{2+} oscillations that were reduced in frequency by 98.4 % ($p< 0.05$) when exposed to carbenoxolone (CBX, 50 μM), a none specific Cx channel inhibitor. Gap27 (200 μM), a Cx mimetic peptide that blocks hemichannel responses (assayed by ATP release and dye uptake) after short incubation, reduced the spiking frequency by 96.4 % ($p< 0.05$). Suramin (200 μM) and PPADS (75 μM), two P2Y receptor antagonists, decreased the spiking frequency by 90.5 % ($p<0.05$) and 96.4% ($p<0.01$) respectively. Apyrase (5 U/ml), an enzyme that rapidly degrades extracellular ATP, reduced the spiking frequency by 71.4 % ($p<0.01$). None of these agents affected the amplitude of the Ca^{2+} oscillations. Both gap27 (56.6 %, $p<0.01$) and CBX (53.4 %, $p<0.05$) reduced the NOR-induced contractions. Incubation with suramin decreased the NOR-induced contractions by 31.6 % ($p<0.001$). Our results suggest a role for Cx hemichannels and purinergic signaling in Ca^{2+} oscillations and contractility.