Smooth muscle action potentials mediate propagating intercellular Ca^{2+} signals and contraction in resistance arteries

Our understanding of the role of vascular gap junctions and intercellular Ca^{2+} signalling and associated contraction in small resistance arteries is somewhat controversial. In the present study, we investigated the temporal and spatial relationship between intercellular Ca^{2+} signals generated by smooth muscle action potentials (APs) and synchronization of contraction in mesenteric resistance arteries (MA). Methods: Wistar rats of either sex were humanely killed in accordance with UK legislation (Schedule 1 procedure; Animals (Scientific Procedure) Act 1986, UK). Second and third order branches of the superior mesenteric artery were dissected carefully and cleaned of adherent tissue. For simultaneous Ca^{2+} imaging and tension experiments, artery segments (~3.5mm long, i.d.150-300µm) loaded with fluo-4 were pulled over 50µm diameter stainless steel rods and fixed to the bottom of a small chamber (0.5 ml) in a microscope stage insert and continuously superfused with Krebs' solution (2-3 ml min^{-1}) via a gravity line. Isometric force was independently measured at each end of the MA segment; and images acquired at 30Hz using a 2x objective and Andor spinning disk imaging system. Two positive pressure perfusion systems lines were used for local application of vasoconstrictors or vasodilators (100µm delivery tips, Digitimer, USA). For simultaneous membrane potential and tension experiments, sharp glass microelectrodes were used to impale individual smooth muscle cells in arteries mounted in a wire myograph. Results: (1) Localized application of 120mM KCl to the downstream end of an artery segment increased intracellular Ca^{2+} and stimulated vasoconstriction only in the vicinity of the KCl (spread up to ~700-1200µm from the point of application). (2) In contrast, if arteries were exposed to the K^+-channel blocker TEA (10mM) followed by the L-type Ca^{2+} channel agonist, BayK8644 (1µM) Ca^{2+} waves associated with phasic contraction propagated from one end of the artery to the other without decay (~3.5mm), whether or not the endothelium was intact. These intercellular Ca^{2+} waves propagated along the vessel wall at an average velocity of 3.11±0.12 mm s^{-1} and a frequency of 0.2 - 2 Hz (n=15). Propagation (but not the generation) of the Ca^{2+} signal was reversibly blocked by the gap junction uncoupler 18-β-glycyrrhetinic acid (20µM, n=7). Local downstream application of the K_{ATP} channel activator cromokalim (20µM) blocked ("fire-walled") the invasion of the stimulated Ca^{2+} waves into the downstream area of the artery (n=7). (3) Action potentials recorded in response to Bay K8644 in the presence of TEA were observed at similar frequencies as Ca^{2+} spikes ranging from 0.2 Hz seen as isolated AP associated with a single phasic contraction to bursts of APs ranging from 0.7 to 2 Hz. The amplitude of force associated with APs or Ca^{2+} spikes was dependent on the frequency of the AP and Ca spikes in a burst. These data suggest Ca^{2+}-based action potentials can be evoked if K^+ channels are blocked, and propagate across vascular smooth muscle cells via gap junctions causing conducted vasoconstriction.