113P CONTROL OF ENDOTHELIAL CELL CALCIUM HANDLING IN RAT MESENTERIC ARTERIES

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Activation of endothelial cells (ECs) is linked to increases in intracellular Ca^{2+} and membrane hyperpolarization (Carter *et al.*, 1994). EC membrane hyperpolarization increases the electrical gradient for Ca^{2+} entry, thus has been hypothesized to control Ca^{2+} entry in these cells. In rat mesenteric arteries EC hyperpolarization to acetylcholine (ACh) is blocked by combined inhibition of intermediate- and small-conductance Ca^{2+} activated K^+ channels (Hinton *et al.*, 2003). The current study examined Ca^{2+} handling following EC activation with ACh and investigated the mechanisms that generate this Ca^{2+} profile.

The role of membrane potential was investigated through EC depolarisation with 35mM K^+, and also the combined blockade of intermediate- and small- conductance Ca^{2+} activated K^+ channels (inhibited by TRAM-34 (Wulff *et al.*, 2000) and apamin respectively). The roles of intra- and extra-cellular calcium were investigated by blocking uptake of Ca^{2+} to intracellular stores (with cyclopiazonic acid (CPA)) and the removal of extracellular Ca^{2+}. The role of protein kinase C was investigated through its inhibition by bisindolylmaleimide (BIS).

Male Wistar rats (200-250g) were killed by cervical dislocation. Sections of third order mesenteric arteries were cannulated and mounted in a pressure myograph, pressurised to 50mmHg and perfused with MOPS at 37°C. ECs were selectively loaded with the Ca^{2+}-sensitive dye, fluo-4 AM, and visualized using a confocal fluorescence microscope. ECs were activated by 300nM ACh, in the presence of either 1μM TRAM-34 and 50nM apamin, 35mM K^+, 10μM CPA, 100nM BIS, or the removal of extracellular Ca^{2+}, and the changes in EC Ca^{2+} monitored for 100s. All experiments were performed in the presence of 100μM L-NAME. Statistical comparisons were performed using one way ANOVA, with Bonferroni's post-test.

In control experiments (n=7) 300nM ACh caused a sustained increase in EC Ca^{2+} (data shown as mean±s.e.mean, 20s following ACh administration, relative intensity =1.50±0.05, 100s following ACh administration, relative intensity =1.31±0.03), and also caused unsynchronised oscillations in EC Ca^{2+}. Removal of extracellular Ca^{2+} (n=7) decreased EC Ca^{2+}, 100s following ACh administration relative intensity =1.04±0.03, and reduced Ca^{2+} oscillations ($p<0.001$) in the sustained phase of the Ca^{2+} profile, whereas the intial Ca^{2+} profile was unaffected. The EC Ca^{2+} profile was not altered by incubation with, 1μM TRAM-34 and 50nM apamin in combination (n=5), 35mM K^+ (n=4) or 100nM BIS (n=4). Incubation with 10μM CPA blocked EC Ca^{2+} oscillations (n=5, $p<0.001$) and initial elevations of EC Ca^{2+} ($p<0.001$)

The Ca^{2+} profile in the sustained phase of activation is dependent on extracellular Ca^{2+}. Oscillations in EC Ca^{2+} are dependent on a functional intracellular Ca^{2+} store, and on the presence of extracellular Ca^{2+} in the sustained phase. EC membrane potential did not affect the Ca^{2+} profile.

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