112P A TECHNIQUE TO INCORPORATE CELL IMPERMEANT COMPOUNDS INTO THE ENDOTHELIUM OF PRESSURIZED MESENTERIC ARTERIES

Simon Mather, Kim A. Dora & Christopher J. Garland, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, UK

A lack of pharmacological tools able to manipulate intracellular signalling selectively in the endothelium, has hampered our ability to unravel the mechanisms by which these cells control artery diameter. Cell permeable molecules have a limited use, as they affect both the endothelial and smooth muscle cells. In an attempt to address this problem, we have adopted a method originally described for cells in culture by Okada *et al* (1982), to introduce large cell impermeant compounds selectively into the endothelium of pressurised rat mesenteric arteries. By changing osmotic pressure, we have succeeded in selectively introducing a number of dyes and antibodies into the endothelial cells of pressurised arteries.

Male Wistar rats (200-250g) were killed by cervical dislocation, and the mesenteric arcade removed. 2mm-long sections of a third order branch of the superior mesenteric artery (i.d 250-300μm) were cannulated and mounted in a pressure myograph (Danish Myotechnology). Vessels were pressurized to 50mmHg and perfused with MOPS at 37^{o}C. The loading protocol was a three stage process, involving 10 minutes hypertonic loading 800mOsM (osmolarity adjusted by addition of sucrose), followed by a hypotonic (180mOsM) and isotonic phase. Endothelial cells were loaded with either carboxyfluorescein, rhodamine-dextran (10 kDa), FITC-IgG or IP_3 receptor (IP_3R) antibodies and visualised using confocal microscopy. Dilator responses to ACh, cyclopiazonic acid (CPA) and levcromakalin, and endothelial cell calcium changes to ACh were then assessed in each tissue. Data expressed as mean ± s.e.m. Statistical comparisons were performed using either one-way ANOVA with Bonferroni's post-test or paired t-test.

The loading protocol resulted in extensive endothelial cell incorporation of all three markers in over 75% of cells. The protocol could be repeated without affecting the structural integrity and functional viability of the endothelium. In preparations contracted with phenylephrine (3μM), the cumulative addition of ACh evoked concentration-dependent dilatation (pEC_{50} 6.8±0.16; n=8) that was unaltered by the loading procedure alone (pEC_{50} 6.8±0.05; n=8). Following the incorporation of IP_3R antibodies a significant rightward shift in the concentration response curve was obtained (pEC_{50} 6.2±0.2, n=6; $P \leq 0.001$). Furthermore, IP_3R antibodies reduced the maximum relaxation to 300nM ACh from 80±3% to 24±4% (n=6; $P \leq 0.001$), and peak increases in endothelial cell calcium from 58±9% to 4±2% (n=4; $P \leq 0.05$).

Our data suggest that the osmotic loading protocol can be used selectively to incorporate large cell impermeable compounds into the endothelium of pressurised resistance arteries. Validation of this technique demonstrates the importance of endothelial cell calcium changes in EDHF mediated vasodilatation in small resistance rat mesenteric arteries.

Okada YC *et al*. (1982) *Cell*. 29, 33-41

SM was supported by a Wellcome Trust Prize Studentship