PC1

Flufenamic acid (FFA) stimulates novel calcium channel activity in human conditionally immortalised podocytes (hCIPs) in a nephrin-dependent manner

R.R. Foster[1], G.I. Welsh[1], S.C. Satchell[1], D.O. Bates[2], P.W. Mathieson[1] and M.A. Saleem[1]

[1]Clinical Science @North Bristol, University of Bristol, Bristol, UK and [2]Department of Physiology, University of Bristol, Bristol, UK

Recent studies have linked mutations in TRPC6 (a non-selective cation channel) with progression of renal disease (1,2). These studies showed that the podocyte, within the glomerulus is the target cell for abnormal calcium signalling. Here we demonstrate a difference in calcium channel activity between the two cell types that form the ultrafiltration barrier within the glomerulus (podocytes and glomerular endothelial cells (GEnCs)). This channel activity is dependent on nephrin, a cell adhesion molecule (and podocyte marker) located at the podocyte slit diaphragm. FFA, which has been suggested to activate TRPC6 whilst non-specifically blocking other cation channels (3) was used to stimulate channel activity. Human conditionally immortalised podocytes (hCIPs), nephrin-deficient (ND) hCIPs and hGEnCs were loaded with 10μM Fura2-AM. They were incubated in buffer containing 200nM (low) or 5mM (high) Ca^{2+} and stimulated with 200μM FFA in the presence or absence of 200nM thapsigargin to deplete intracellular calcium ($[Ca^{2+}]_i$) stores, in varying amounts of FCS. Changes in $[Ca^{2+}]_i$ were measured using the normalised fluorescence intensity ratio at excitation wavelengths of 340nm to 380nm (R_{norm}). FFA increased the R_{norm} in hCIPs in the presence and absence of a Ca^{2+} concentration gradient (5mM $[Ca^{2+}]_o$ 1.44±0.11 fold increase, $p<0.01$, n=7; 200nM $[Ca^{2+}]_o$; 1.58±0.01 fold increase, $p<0.01$, n=6 unpaired t-tests), which was significantly reduced when $[Ca^{2+}]_i$ stores were depleted (post-thapsigargin 1.08±0.06 fold increase, $p<0.05$, n=6). However, the presence of FCS post-thapsigargin restored the FFA induced increase in R_{norm} (1.78±0.15 fold increase, $p<0.01$, n=6 unpaired t-test). In contrast the response to FFA in hGEnCs post-thapsigargin was not blocked (2.01±0.12 fold increase, $p<0.01$ paired t-test, n=4). Similarly, in the absence of nephrin (NDhCIPs) the response to FFA was not blocked post-thapsigargin (1.6±0.06 fold increase, $p<0.01$, n=4, paired t-test). The post-thapsigargin response to FFA was FCS dose dependent. In conclusion in hCIPs the FFA induced increase in $[Ca^{2+}]_i$ was dependent on thapsigargin-sensitive $[Ca^{2+}]_i$ stores, except in the presence of FCS whereby it became store independent in an FCS dose-dependent manner. $[Ca^{2+}]_i$ store-dependent activation by FFA was also nephrin dependent and was not seen in hGEnCs. This evidence suggests novel activity of a not-yet specified calcium channel in hCIPs, which is nephrin and FCS dependent and potentially involved in progression of renal disease.

Winn MP et al. (2005). Science 308, 1801-4.

Reiser J et al. (2005). Nat Genet 37, 739-44.

Inoue R et al. (2001). Circ Res 88, 325-32.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC2

Characterization of the TRPM8 calcium channel in rat aorta and tail artery

D. Melanaphy[1], C.D. Johnson[1], D. Paula[1], P. Anderson[2] and Z. Alexander[1]

[1]Cell and Metabolic Signalling Research Group, Queen's University, Belfast, UK and [2]Respiratory Research Group, Queen's University, Belfast, UK

Ca^{2+}-permeable cation channels not only control excitability of vascular myocytes but also form an important Ca^{2+} channeling route. Several mammalian homologues of Drosophila transient receptor potential (TRP) proteins are strong candidates for such channels (Beech, 2005). Recent studies of novel melastatin TRPM8 channels have focused on their role in cold transduction in sensory neurones. However, there are now reports that these receptors are present in blood vessels and may be active in vascular tone (Yang et al., 2006). We have examined this possibility in detail using functional and molecular techniques.

Vascular tissue was taken from humanely-dispatched Wistar rats (12 weeks). Sections of precontracted proximal tail artery and thoracic aorta with endothelia removed were mounted in Krebs for isometric contraction study. Vasoconstrictions induced by KCl (60 mM; tail: 0.85±0.13 g, n=7; aorta: 0.88±0.09 g, n=6) were inhibited (paired Student's t-test, performed on absolute data) by addition of selective TRPM8 channel agonists, menthol (300 μM; tail: 43±9 % control, $P< 0.01$; aorta: 61±10 %, $P< 0.01$) or icilin (50 μM; tail: 39±16 % control, n=3, $P< 0.05$; aorta: 42±7 %, n=3, $P< 0.05$). Similar results were obtained with contractions evoked by noradrenaline (2 μM). Sympathetically-evoked constrictions (0.6±0.32 g, n=3) in tail artery were significantly reduced by menthol (56±9 % control, $P< 0.05$).

Single vascular smooth muscle cells (VSMCs) were isolated from proximal tail artery for patch-clamp recordings. With high K^+ (125 mM) and low intracellular Ca^{2+} buffering (0.3 mM EGTA in the pipette solution), STOCs discharge (reflecting BKCa channel activation due to spontaneous localised Ca^{2+} release events) was observed at 1.4-4.4 Hz at holding potentials from -40 to -10 mV. These STOCs were significantly accelerated by 100 μM menthol application (75±19% frequency increase, $P<0.01$, n=4), while the amplitude of these currents was markedly reduced. Eventually (1-2 min after menthol application) STOCs discharge was terminated.

PCR studies in VSMCs from thoracic aorta, tail, femoral, renal and mesenteric arteries with endothelia removed all showed the presence of mRNA for TRPM8 receptor proteins.

We conclude that TRPM8 channels are present and functional in a range of different blood vessels. Since they can be localised not only in the plasma membrane, but also in the SR membrane (Abeele et al., 2006) Ca^{2+} store release and depletion evidenced by STOCs initial acceleration and eventual suppression seems the most likely mechanism of vasoconstrictor inhibition by TRPM8 agonists.

Abeele FV et al. (2006). J Biol Chem 281, 40174-40182.

Beech D (2005). Clin Exp Pharmacol Phys 32, 597-603.

Yang XR et al. (2006). Am J Physiol Cell Mol Physiol 290, L1267-76.

We thank the Faculty of Medicine and Health Sciences, Queen's University of Belfast, for support.