Sub-urothelial $KCNQ^+$ interstitial cells modulate peristalsis in the mouse renal pelvis

M.J. Nguyen,[1] K. Ohta,[2] J. Iqbal,[1] H. Hashitani,[3] K.I. Nakamura[2] and R.J. Lang,[1] [1]Department of Physiology, School of Biomedical Sciences, Monash Univiersity, Clayton, VIC 3800, Australia, [2]Department of Anatomy, Kurume University of School of Medicine, Kurume 830-0011 Japan and [3]Department of Cell Physiology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan.

In the last three decades, evidence has accumulated that the pacemaker cells driving peristaltic contractions in the renal pelvis (pelviureteric peristalsis) are specialized smooth muscle cells (SMCs) called *'atypical'* SMCs (ASMCs). However, it is now apparent that recent 'Ca^{2+} oscillator' models of autorhythmicity in these ASMCs aren't sufficient to describe the generation and propagation of the pacemaker potentials and the action potentials they trigger in neighbouring typical SMCs (TSMCs). Interstitial cells (ICs) expressing K_V7 (KCNQ) channel currents and immuno-reactivity located in sub-urothelial regions adjacent to the TSMC wall appear to fundamentally modulate pelviureteric peristalsis. In this study we aimed to further elucidate the structure and function of K_V7/$KCNQ^+$ ICs in maintaining pelviureteric peristalsis.

The kidneys and attached ureters were removed from Balbc or Swiss outbred mice killed by cervical dislocation and exsanguination under isoflurane anesthesia. The renal pelvis was dissected free of its surrounding parenchyma, fat and adventitia and prepared for experimentation. Previous immnunohistochemisical analysis has revealed that a layer of $KCNQ5^+$ ICs, which were negative for Kit or α-smooth muscle actin, located in the sub-urothelium space; their number also increased with distance from the base of the papilla as the urothelium transitions from a double-layered squamous epithelium to a more complex transitional epithelium (Iqbal *et al.*, 2012). Three dimensional reconstructions of TSMCs, ASMCs and these sub-urothelial ICs from 600-900 serial sections (100 nm thick) of the proximal region of the mouse renal pelvis imaged with a Focused Ion Beam Scanning Electron Microscope (FIB SEM) revealed that TSMCs lie in close apposition to both ASMCs and the sub-urothelial ICs. In contrast, macrophages and fibroblasts appear not to make any close appositions with any others cells within the region.

Spontaneous changes in the diameter of the renal pelvis were recorded with a video camera and analyzed with Diamtrak software. Application of 'non-selective' K_V7 channel subunit blockers (Xe991, linopirdine 10 μM) and activators (flupirtine 10 μM) increased and decreased, respectively, the frequency of muscle wall contraction. However, the inhibitory effects of linopirdine were only evident after capsaicin depletion of primary sensory afferents. The frequency and amplitude of Ca^{2+} transients in fluo-4 loaded TSMCs were also significantly increased and decreased, respectively, by Xe991; while only the amplitude and integral of these transients were reduced by flupirtine. Interestingly, both flupirtine and Xe991 significantly decreased the frequency, amplitude and integral of the spontaneous Ca^{2+} transients in ASMCs.

In order to ascertain which subunits of $K_V7.x$ may be functional in the renal pelvis the effects of 3 selective openers of expressed homomeric $K_V7.x$ subunit channels were examined. L-364,373 (10 μM n = 8), a selective opener of of $K_V7.1$ subunit channels significantly decreased the amplitude and ½ width of the spontaneous contractions of the renal pelvis. However the integral and frequency of these contractions were not significantly different from control ($p > 0.05$). The selective activation of $K_V7.2$ subunits with ML-213 (230 nM n = 12) significantly decreased the amplitude, integral and frequency of renal pelvis contractions. Similar significant decreases in these parameters were observed when $K_V7.2$ and $K_V7.4$ subunits were activated with ML-213 (510 nM n = 8). The subsequent addition of XE991 (10 μM) in the presence of ML-213 (230 or 510 nM) or L-364,373 (n = 7) significantly reversed the effects of these agents on contraction frequency. In contrast, ICA069673 (1 μM n = 12), a selective opener of $K_V72/3$ subunit channels, did not significantly alter from control the amplitude, ½ width, integral or frequency (all $P > 0.05$) of renal pelvis contractions. However, the subsequent addition of XE991 (10 μM) in the presence of ICA069673 still significantly increased contraction frequency.

As activators of $K_V7.2/4$ subunit channels selectively reduced the frequency of spontaneous contractions in the renal pelvis and that Xe991-sensitive K_V7 currents have only been recorded in ICs (Iqbal *et al.*, 2012) and not ASMCs nor TSMCs, we suggest that these sub-urothelial ICs directly modulate the conduction of excitation along the distal renal pelvis wall, particularly at the ureteropelvic junction. As such, manipulation of $KCNQ^+$ IC function may prove a useful therapeutic in the treatment of *in utero* or peri-natal congenital obstructive hydronephrosis.

Iqbal J, Tonta MA., Mitsui R, Li Q, Kett MM, Li J, Parkington HC, Hashitani H. & Lang RJ. (2012) *British Journal of Pharmacology* **165,** 2389-408.