**Kv7 Channel activation reduces spontaneous excitability of mouse isolated detrusor smooth muscle, whilst leaving stimulus-evoked contractions unaffected.**

Peter Sidaway¹, Jessica Bowen¹, Francesca Caputo¹,², Keith Brain¹

¹University of Birmingham, Medical School B1527T, UK, ²University of Siena, Department of Neuroscience S3100, Italy

Introduction: Overactive bladder syndrome (OABS) leads to incontinence, typically as a result of spontaneous urinary bladder smooth muscle (UBSM) activity during the filling phase.

Methods: Male Balb/C mice were humanely killed in line with the UK Animals (Scientific Procedures) Act 1986 and European Communities Council Directive 86/09/EEC. Electrophysiology: UBSM strips (1-2 x 4-6mm) were pinned to a Sylgard-lined organ bath. Spontaneous changes in intracellular resting membrane potential (RMP) were recorded. Contraction assay: UBSM strips were tensioned to 9.8 mN in a modular organ bath. Responses to electrical field stimulation (EFS) were monitored in the absence and presence of drug treatments. 

Ca²⁺ imaging: UBSM strips (1-2 x 4-6mm) were loaded with the Ca²⁺ indicator Oregon Green 488 BAPTA 1-AM. Spontaneous calcium transients in the absence and presence of drug treatment were monitored using a fluorescence microscope with a 465nm LED light source.

Results: Flupirtine (10µM), a Kv7.2-7.5 channel activator, significantly inhibited spontaneous action potentials (sAPs) (0.03 ± 0.03 min⁻¹) compared with control recordings (0.6 ± 0.3 min⁻¹, N=4 bladders, Mann-Whitney test p<0.05). In the presence of flupirtine, there were no significant effects upon mean RMP (-46.1 ± 1mV) compared with controls (-45.1 ± 2.2mV). Where changes in intracellular Ca²⁺ were monitored, flupirtine (10µM) significantly inhibited spontaneous WCTs (1.1 ± 0.4 min⁻¹) compared with controls (4.1 ± 1.5 min⁻¹ N=4, Wilcoxon’s matched pairs test, p<0.05). WCTs, which are the optical correlate of smooth muscle sAPs (Young et al., 2008), apparently occurred at a higher frequency than sAPs; this difference is attributed primarily to an inherent bias to record electrically from relatively inactive cells due to the difficulty of maintaining recordings in highly-active cells. XE991 (10µM), an inhibitor of Kv7.2-7.5 channels significantly increased the frequency of WCTs (5.7 ± 1.6 min⁻¹) compared with controls (0.4 ± 0.3 min⁻¹ N=3 Wilcoxon’s matched pairs test, p<0.05), suggesting that Kv7.2-7.5 channel activity is an important regulator of UBSM cell spontaneous excitability. EFS-induced contraction of isolated UBSM strips in the presence of a range of flupirtine concentrations (0.3-30 µM) did not show any statistically significant changes compared with controls (one way ANOVA, Bonferroni’s post-hoc test, p>0.05). Where XE991 was applied to contraction assays, there was a statistically significant increase in EFS-induced force of contraction at concentrations ≥3µM (one way ANOVA, Bonferroni’s post-hoc test, p<0.05) but not smooth muscle tone.

Conclusions: Activation of Kv7.2-5 channels appears to regulate spontaneous UBSM activity whilst having no significant effects upon either RMP, or EFS-induced contractions. These findings suggest that bladder Kv7 channels are a promising target for the treatment of OABS, as channel activation affects spontaneous but not neuronally-evoked, activity.