M2 receptor activation modulates human and guinea pig detrusor contractions via cAMP inhibition

S Bishara1, J Shah1, B King, J2 Malone-Lee3, CH Fry1.1 Department of Surgery, University College London (UCL) 2 Department of Physiology (Hampstead Campus) UCL 3 Department of Medicine (Archway Campus) UCL

Human detrusor contraction is mediated principally by acetylcholine released from parasympathetic nerve terminals. Anticholinergic drugs remain the most common treatment for detrusor overactivity. Across a range of mammalian species, it has been demonstrated that detrusor contraction is mediated by M3 acetylcholine receptors. The more populous M2 receptor is believed to have a regulatory role - although this remains unclear. We have measured the force of contraction generated by muscarinic agonists in human and guinea pig detrusor tissue in the absence then presence of muscarinic M2 antagonists, to determine the relative contribution of M2 receptor signalling in these tissues.

Human detrusor tissue was obtained with consent from twelve patients undergoing open bladder surgery and guinea pig tissue was obtained from freshly isolated bladders. Muscle strips were mounted in an organ bath and superfused with a balanced salt solution. The force of contraction was measured in response to varying concentrations of carbachol and oxotremorine or to electrical field stimulation (EFS) ranging from 1Hz to 60 Hz (47.5 V, 3 second train, 0.1 millisecond pulse width), in the absence and then presence of the M2 antagonist methoctramine. Data are expressed as mean ± SD. Differences between groups were established using student’s t test and the null hypothesis was rejected when p < 0.05.

In human detrusor preparations, carbachol and oxotremorine evoked contractions with indistinguishable potency (pEC50 5.8 ± 0.4 (n=15) and 6.1 ± 0.51 (n=7) respectively). There was significant M2 receptor activity within all human detrusor strips; pKB values for methoctramine were 8.1±0.3 (n=12) and 8.3 ±0.7 (n=7) against carbachol and oxotremorine respectively. Methoctramine (10 nM) produced a mean reduction in force of 40% ±21.1% at 16 Hz EFS (n=5, p<0.05). With guinea pig detrusor, oxotremorine was a significantly more potent agonist than carbachol; (pEC50 = 6.39 ±0.53 (n=7) and pEC50 =5.34 ±0.29 (n=19) respectively, p<0.05). Significant M2 receptor signalling was observed when oxotremorine, but not carbachol, was used as an agonist and this was antagonised by methoctramine (pKB=8.57 ± 0.45, n=7). Forskolin (10 uM) inhibited oxotremorine contractions indistinguishably from 10nM methoctramine (dose ratio =2.38 (95% CI = 1.95-3.23) and dose ratio = 2.93, (95% CI = 1.85-4.01), respectively, n=5) but, like methoctramine, had no effect on carbachol induced contractions.

These results indicate that there is significant M2 receptor activity in both human and guinea pig detrusor. This activity is likely to be mediated by cAMP inhibition. In the guinea pig, unlike human detrusor, M2 activity could not be demonstrated when carbachol was used as an agonist and this disparity may be due to subtle structural and pharmacological differences in M2 receptors between these two species. The human data suggests that some patients may benefit from the targeted inhibition of the M2 receptor in addition to the M3 receptor. Further elucidation of cAMP dependent pathway in human tissue may lead to novel therapeutic targets.