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Authors: R Sakakibara, T Uchiyama, T Hattori, K Yasuda*, R Chess-Williams**, CR Chapple**
Institution: Chiba University, Dokkyo University*, Sheffield University**
Title: THE ROLE OF M2-MUSCARINIC RECEPTOR SUBTYPES MEDIATING CONTRACTION OF THE CIRCULAR AND LONGITUDINAL SMOOTH MUSCLE OF THE PIG PROXIMAL URETHRA

Aims of study

It has been reported that contraction of urinary bladder body is mediated via a minor population of M_3-receptors. Although no direct contractile response to M_2 receptor activation can be demonstrated, an indirect influence on contraction via inhibition of cAMP- mediated smooth muscle relaxation has been reported. However there is little information available regarding the functional specificity of muscarinic receptors in the urethra. The present study investigates the characterisation of muscarinic receptor subtypes in the circular and longitudinal smooth muscle of the pig proximal urethra in normal tissues and in tissues following cyclic AMP elevation.

Methods

In receptor binding studies, displacement experiments using [^{3}H]QNB with 4-DAMP (M_3-selective antagonist) and methoctramine (M_2-selective antagonist) determined the presence of M_2 and M_3 receptors. In the functional studies *in vitro,* the affinity of these antagonists against carbachol induced contractions of tissue strips were calculated in normal tissues and tissues following cyclic AMP elevation (precontraction with KCl and relaxation with isoprenaline).

Results

In saturation binding studies (n=4), receptor density was 44.1±13.2 fmol/ mg protein and the dissociation constant (Kd) for [^{3}H] QNB was 0.26 nM. Displacement of [^{3}H] QNB by 4-DAMP (n=8) and methoctramine (n=4) best fitted a one-site model with Hill's slopes close to unity, the affinity (pKi) values indicating the presence of M_2 receptors.

In normal circular and longitudinal muscle strips, the maximum contraction, compared with that to 50mM KCl was larger in longitudinal muscles (99.8%) than in the circular muscles (49.4%). In cAMP-elevated tissues, this was increased in circular muscles (74.7%), but did not increase significantly in longitudinal muscles (103.3±12.0%). On normal circular strips *in vitro* (n=18), 4-DAMP had a high affinity (pK_B=9.30) without affecting maximum responses, but the Schild slope was less than unity (0.76). Methoctramine competitively antagonized responses to carbachol with a pK_B value of 6.90 (n=15). On normal longitudinal muscle strips, 4-DAMP (n=20) and methoctramine (n=12) produced surmountable antagonism of responses to carbachol, and yielded mean pK_B values of 8.98 and 6.22 with Schild slopes not significantly different from unity.

Following cyclic AMP-elevation in circular muscles, pK_B values for 4-DAMP (8.7) were significantly less ($p=0.0015$), and those for methoctramine (pK_B=7.3) were significantly greater (p=0193) than in normal tissues. Schild slopes for 4-DAMP were not different from unity but those for methoctramine (0.68) were significantly ($p<0.0001$) less than unity.

In longitudinal muscle strips following cAMP-elevation, 4-DAMP (n=25) and methoctramine (n=12) competitively antagonised carbachol responses with Schild slopes close to unity, pK_B values for 4-DAMP (8.78) were not significantly different from values for normal tissues, but those for methoctramine (6.92) were significantly ($p<0.0001$) greater than in the normal tissues.

Conclusions

The pig urethra appears to have predominantly M_2 receptors. Contraction of the normal urethra appears to be mediated by M_2 and M_3 -receptors in circular muscles, but by solely M_3 -receptors in longitudinal muscles. Following cAMP-elevation, the contribution by M_2 -receptors appeared to be demonstrated in both tissues, but the involvement of M_2 -receptors appeared greater in circular muscles.