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Title (type in CAPITAL LETTERS): THE ROLE OF M2-MUSCARINIC RECEPTORS IN PIG CONTRACTION

Aims of Study:Several studies have identified the M3-receptor as being the predominant muscarinic receptor subtype responsible for contraction of the bladder to muscarinic agonists in vitro. However in many species the density of the M2-receptor subtype is greater than that of the M3-subtype. Recently it has been shown that in the rat bladder, where the M2:M3 ratio is about 9:1, an M2-mediated contraction can be demonstrated following M3-receptor inactivation and elevation of cAMP levels (re-contraction).[1] This study examines whether a similar role for M2-receptors can be demonstrated in the pig bladder, where the M2:M3 ratio is closer to that reported for the human bladder (i.e.3:1) .

Methods:Pig detrusor strips (dome region) were set up in aerated Krebs solution at 37°C and concentration-response curves (CRCs) obtained to carbachol in the absense and presence of the M3-selective antagonist 4-DAMP and M2-selective antagonist methoctramine. Similar experiments were performed on tissues following selective M3-inactivation (incubation of tissues with 40nM 4DAMP mustard for 60 min in the presence of 1μM methoctramine to protect M2-receptors), precontraction with 50mM KCL and relaxation with isoprenaline (30μM) or forsklin (1μM). Parallel control experiments were used to correct for time-dependent changes in tissue sensitivity. Antagonist affinity (PKB values) and Schild plots were constructed from shifts of CRCs to carbachol.

Results:On normal detrusor muscle strips in vitro, 4-DAMP and methoctramine caused parallel rightward shifts of CRCs without a change in maximum responses, and yielded a mean PKB value of 9.6 (n=4) and 6.1(n=6), respectively, and a Schild slope similar to unity (0.94±0.12 and 0.89±0.15, respectively). In tissues where the M3-receptors had been inactivated and cAMP levels elevated, a re-contraction was obtained to carbachol. 4DAMP again caused rightward shifts of CRCs, but the antagonist was less potent, the mean PKB value being 8.72(n=9) significantly lower ($p<0.01$) than in the normal tissues. Methoctramine also caused rightward shifts of CRCs, and the antagonist was more potent, the mean PKB value being 7.06(n=6) significantly higher ($p<0.001$) than in the normal tissues.

Conclusions: The data in normal tissues suggest that in the pig, as in other species, responses were mediated solely via the M3-receptor. The re-contraction after M3-inactivation with protection of M2-receptor appears to be mediated by M2-receptor.

Table: Effects of antagonists on CRCs obtained to carbachol

Antagonist	Tissue	Number	PKB	slope
4DAMP	Normal	n=4	9.6	0.94±0.12
4DAMP	M3-inactivation (M2-protection)	n=9	8.7	1.17±0.09
Methoctramine	Normal	n=6	6.1	0.89±0.15
Methoctramine	M3-inactivation (M2-protection)	n=6	7.1	0.91±0.20

Reference: Hedge SS et al.,(1997)Br.J.Pharmachol 120,1409-1418.