affinity estimates (pK_b). Table 1 contains pK_b values from the present study and the pK_i and pA_2 values previously obtained for cloned receptors using radioligand binding and guinea pig paced atria (M_2), respectively. The data from the current study are consistent with reported binding values for the human cloned M_2 mAChR as well as functional values obtained from the guinea pig paced atria (M_2) tissue bath assay [4] and demonstrate the existence of functionally coupled muscarinic receptors in cultured bladder smooth muscle cells possessing M_2 mAChR pharmacology.

Table1.

Drug	M_1	M_2	HBD	M_3	M_4	M_5	GPA
	pK_i	pK_i	pK_b	pK_i	pK_i	pK_i	pA_2
Pirenzepine	8.0	6.3	<6.0	6.8	7.1	6.9	6.8
Darifenacin	7.8	7.0	<6.8	8.8	7.7	8.0	6.9
AQ-RA 741	7.6	8.9	8.7	7.5	8.0	6.0	8.3
PD 102807	5.5	5.9	6.1	6.7	7.4	5.5	5.5
MT-3	6.5	<5.4	<5.0	<5.4	8.2	5.9	NT
Himbacine	6.7	8.0	8.2	6.9	7.8	6.1	8.1
Tripitramine	8.5	9.4	9.0	7.1	8.0	7.3	9.7

Table 1. Mean affinity estimates ($n \geq 3$) from radioligand binding (pK_i), cyclic AMP accumulation human bladder detrusor (pK_b) and guinea pig paced atria (pA_2).

Conclusions:

The pharmacological profile of the muscarinic receptor-mediated inhibition of adenylyl cyclase in the cultured smooth muscle cells correlates best to the binding profile of the human M_2 mAChR expressed in the CHO cell, as well as, the "classical" M_2 mAChR represented by the guinea pig paced atria. Demonstration of a functional role for the M_2 mAChR combined with the relative abundance of the receptor in the bladder of many species, suggests that antagonism of both M_2 and M_3 mAChRs may be beneficial for the treatment of overactive bladder conditions.

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THE EFFFECTS OF CHRONIC IN VIVO ATROPINE TREATMENT ON URINARY BLADDER FUNCTION IN THE GUINEA-PIG

Aim of Study:

Despite the substantial increase in knowledge concerning the normal control of micturition and the lower urinary tract and the pathology underlying urge incontinence associated with detrusor instability, there are as yet no effective pharmacological treatments available; antimuscarinic compounds remaining the most widely used drug thearpies. However, the dogma through which such treatment was originally conceived (namely that instability was the result of an overactive cholinergic motor input to the bladder) has recently been questioned with the finding that in many unstable bladder samples cholinergic denervation was evident. Additionally few studies have attempted to elucidate any potential bladder changes which may occur as a result of antimuscarinic therapy. Presently we have

investigated the effect of chronic in vivo atropine treatment on the in vivo and in vitro properties of guinea-pig urinary bladder.

Methods:

Female guinea-pigs (400g) were surgically anaesthetised using a combination of hypnorm (midazolam) and hypnovel (fentanyl citrate and fluanisone) and Alzet® osmotic mini-pumps (model 2ML4, Alza Corp., CA, U.S.A.) implanted subcutaneously between the scapulae to allow continous delivery of atropine at a rate of 0.2 mg/Kg/day. Animals were allowed to recover and housed in groups for 14 or 28 days post operation, controls consisted of age matched non-operated animals. At 14 and 28 days animals underwent urodynamic investigation under urethane anaesthesia using a constant infusion pump (flow rate 0.25 ml/min), intra-vesical pressure was recorded throughout. Voiding occurred into a beaker placed on a balance, the output of which was recorded electronically. Residual volume was measured by gentle pressure on the abdomen to express the bladder contents onto the beaker/balance. On completion the animal was sacrificed and the bladder removed, cleared of adhering connective tissue and weighed. In addition bladder tissue was taken from atropine treated dissection microscope. Strips were placed in 0.2 ml organ baths continually perfused with carboxygenated Krebs' solution (37°C) and initially raised to a tension of 1g. An equilibration time of 60 minutes was allowed prior to experimentation subsequent to which strips were challenged to a variety of agonists (carbachol, ATP, Krebs' solution with increased KCl content) and to electrical stimulation of intrinsic nerves. Isometric tension changes were measured using Pioden force displacement transducers and recorded electronically. Tension changes are expressed as g of tension per mg of tissue weight. Statistical analysis was carried out using Student t test and ANOVA, a figure of $p<0.05$ was considered significant.

Results:

No significant differences in body weight were found between 14 and 28 day treatment groups and controls. Bladder weight was significantly ($P<0.01$) increased in the 28 day treatment group when compared to controls. Urodynamic investigation revealed substantial changes in voiding properties, residual volume was significantly ($p<0.05$) increased in both treatment groups (14 day-1.42±0.57 ml, 28 day-2.56±0.55 ml, controls 0.24±0.24 ml). In addition threshold and voiding pressure were decreased and total bladder capacity increased in both 14 and 28 day atropine treated animals compared to controls.

In vitro investigation of detrusor smooth muscle strips similarly revealed changes in experimental animals. Responses to carbachol (1.08±0.22, 0.58±0.16 and 0.62±0.15 g/mg for control, 14 and 28 day groups), to electrical stimulation of intrinsic nerves (0.92±0.11, 0.42±0.08 and 0.23±0.07 g/mg for control, 14 and 28 day groups) and to direct depolarisation in high K+ Krebs solution (1.05±0.12, 0.71±0.15 and 0.53±0.1 g/mg for control, 14 and 28 day groups) were significantly ($p<0.01$) reduced in atropine treated animals. No significant difference was found in the response to ATP between the groups.

Conclusion:

The present investigation suggests that chronic exposure to antimuscarinic agents can significantly alter the in vivo voiding properties in guinea-pigs, as well as having effects on the physiological properties of the detrusor smooth muscle itself. Further studies are required using clinically utilised antimuscarinic compounds at clincal doses inorder to varify these findings. The finding that atropine treated animals appear to have significantly increased bladder capacity and residual volume may be important in view of