significance of K_V channels was investigated using strips of rabbit detrusor. The detrusor was cut into strips 12 x 2 mm and the mucosa and serosa were removed. Using isometric tension recording the response of strips to 3,4-diaminopyridine (3,4 DAP), a specific blocker of K_V channels was investigated.

RESULTS: Detrusor stained positive for $K_V1.3$ and $K_V1.6$. Staining was clearly reduced if the antibodies were preadsorbed with the respective antigenic peptide (10μM). Western blotting confirmed the presence of protein bands at the appropriate weights. 3,4 DAP (0.1-1mM) increased the baseline tension of detrusor strips, and increased the frequency of spontaneous contractions. Blockers of BKCa channels (iberiotoxin or penitrem A) or K_{ATP} channels (glibenclamide) were ineffective.

CONCLUSIONS: K_V channels have a marked functional role in the maintenance of the resting tension and oscillation contractions of detrusor muscle. Of these K_V channels we have demonstrated that $K_V1.3$ and $K_V1.6$ are present in smooth muscle cells of stable human bladder. These channel subunits may provide a molecular mechanism for the regulation of bladder stability, and a novel target for pharmacological manipulation in the treatment of detrusor instability.

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CHARACTERIZATION OF THE MUSCARINIC CHOLINOCEPTOR-MEDIATED INHIBITION OF ADENYLYL CYCLASE IN PRIMARY CULTURED HUMAN BLADDER SMOOTH MUSCLE CELLS.

Aims of Study:

The muscarinic cholinoceptor (mAChR) family is currently comprised of five pharmacologically and molecularly defined receptors, M_1-M_5. mAChRs are known to play a prominent role in bladder contraction (voiding) and mAChR antagonists, such as oxybutynin, have been used to treat overactive bladder. Radioligand binding and immunoprecipitation studies have demonstrated the existence of both M_2 and M_3 mAChRs at a ratio of 3:1 (M_2:M_3) in human bladder detrusor [1]. Functional tissue bath contraction studies using human bladder strips have consistently demonstrated the importance of M_3 mAChRs, acting via activation of phospholipase C (PLC). More recently, the role of the M_2 mAChR in reversing β-adrenoceptor mediated relaxation ("recontraction"), thought to be mediated through inhibition of adenylyl cyclase, has been described in the rat bladder [2]. The M_3 mAChR-mediated response has also been demonstrated in primary cultured human detrusor smooth muscle cells with robust increases in inositol phosphates accumulation in response to carbachol and antagonist affinities consistent with contraction studies [3]. The current study characterized the muscarinic receptor mediating the carbachol-induced inhibition of forskolin-stimulated cyclic AMP accumulation in primary cultured smooth muscle cells prepared from three patients using seven muscarinic antagonists.

Methods:

Human bladder detrusor smooth muscle cells (HBD) were cultured in tissue culture flasks (T-162) containing minimum essential medium (MEM), 10% fetal bovine serum (FBS) and penicillin/streptomycin/fungizone (P/S/F) at 37°C in 7% CO_2. Cells were obtained from three separate sources: commercial source, academic source and in-house preparation. The cells from all three patients were examined between passages #5 and #11.

The methods used for determination of cAMP accumulation were of two types, a single column chromatographic separation of [^{3}H]-cAMP from [^{3}H]-adenine and [^{3}H]-ATP and a commercially available 96-well format cyclic AMP assay kit. Liquid scintillation spectroscopy was used to determine DPM for both methods used.

Results:

Carbachol was shown to inhibit forskolin-stimulated cAMP accumulation with a pEC_{50} value of 6.3. Seven antagonists with different subtype affinities were examined using inhibition curves and the Cheng-Prusoff [5] equation to determine

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