respectively. The contractile responses and releases of ACh and ATP induced by EFS were significantly inhibited by the pretreatment with tetrodotoxin (1 μM). ATP release during EFS increased with age, and ACh release decreased with age. There was a significant positive correlation between age and ATP release, and there was a significant negative correlation between age and ACh release (figure).

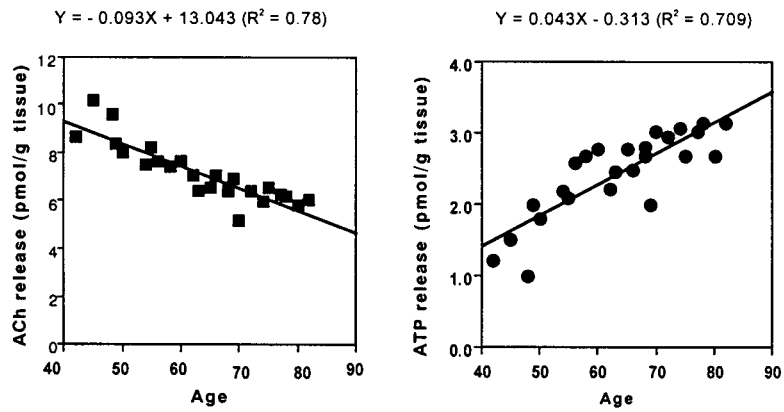

Figure: Correlation between age and ACh release (left) and ATP release (right) in human bladder

Conclusions

The present data suggest that there are age-related increase in ATP release and decrease in ACh release during EFS. This may contribute to age-related functional changes in human bladder smooth muscles.

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VOLTAGE GATED POTASSIUM CHANNELS IN DETRUSOR MUSCLE

AIMS OF STUDY: The aetiology of detrusor instability remains unknown, but myogenic change is thought to be important (1). Voltage-gated potassium channels (K_V channels) are important in the repolarising phase of action potentials and in the control of membrane potential. Potassium channel dysfunction may have an aetiological role in detrusor instability and may offer the possibility of a new pharmacological target in the treatment of detrusor instability.We have investigated the function of K_V channels and the expression of six subunits from the *Shaker*-related K_V1 family in bladder smooth muscle.

METHODS: The expression of the six K_V1 channel subunits was investigated in stable human detrusor using tissue obtained from cystectomy specimens. Slices of detrusor were labelled with channel-specific polyclonal antibodies and a double labelling immunofluoresence protocol was used to compare channel localisation with that of smooth muscle α-actin. Images were collected using a cooled CCD camera and processed using Improvision Openlab software. The specificity of the antibodies was confirmed by Western blotting. The functional

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.

ACKNOWLEDGEMENTS: The K_V1 antibodies were a gift from Dr. HG Knaus, University of Innsbruck

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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