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MUSCARINIC RECEPTORS 1-5 MRNA IN UROTHELIUM: DIFFERENTIAL EXPRESSION IN TWO RAT MODELS OF NEUROGENIC BLADDER

Hypothesis / aims of study

The mechanism of action of anti-cholinergic drugs for overactive bladder (OAB) has historically been considered a specific effect on muscarinic receptors (MRs) of the detrusor layer. Recent work has used immunohistochemistry to demonstrate M_2 receptors on human urothelium that are closely associated with sensory nerves. Furthermore, an as yet uncharacterized diffusible urothelium-derived inhibitory factor (UDIF) which antagonizes cholinergic associated detrusor contraction has been proposed.
Spinal cord injury (SCI) and Diabetes (DM) alter bladder physiology to induce variable hypocontractile and overactive bladder states. Changes in MR expression likely play an important role in these bladder alterations, yet a detailed comparison of MR transcripts in SCI and DM has not been performed.
The aim of our study was to evaluate the existence of MR subtypes mRNA in human urothelium and determine the differential expression of each MR subtype in the bladders of SCI and DM rat models.

Study design, materials and methods

Human bladder tissue was obtained from the posterior bladder neck of three radical retropubic prostatectomy patients, and separation of urothelium from detrusor layers was performed using microdissection techniques. Good separation of bladder layers was assured by H&E histochemistry.
Nine female Sprague-Dawley rats underwent spinal cord transection at the T 8-9 level and three were sacrificed at each time point (7 days, 14 days, and 21-28 days post-spinalization). Their bladders were collected as well as bladders from three normal controls. Four rats had DM (blood glucose ≥ 350 mg/dl) induced by a single intra-peritoneal injection of streptozotocin (65 mg/kg) and were sacrificed either at 26 days or 10 weeks.
Total RNA was extracted from each tissue and subsequent reverse transcription-polymerase chain reaction was carried out using species-specific primers for the five muscarinic subtypes (M_{1-5}) and GAPDH as an internal loading control. cDNA amount and cycle number were adjusted to render submaximal amplification for all genes analyzed.

Results

mRNA from all five muscarinic subtypes was detected in human urothelium. M_2 and M_3 transcripts were demonstrated in detrusor tissue while M1 and M4 mRNA expression in detrusor was variable. M5 mRNA was not detected in detrusor tissue.
Transcripts for all five MR subtypes were detected in the bladders of pathologic and control animals. In SCI bladders, M_1 mRNA was significantly downregulated ($p=0.012$) and M_4 and M_5 showed a trend of decreased expression compared to normal controls. On the contrary, in DM rats M_1 mRNA expression was unchanged while M_4 and M_5 mRNA was significantly lower than controls ($p=0.026$ and 0.021 respectively). M_2 and M_3 mRNA expression was unchanged in both models compared to normal controls.

Interpretation of results

The presence of multiple subtypes of MR mRNA in human urothelium has previously not been reported. These receptors may have a role in sensory modulation and affect release of the proposed but as of yet uncharacterized UDIF, a significant antagonist of detrusor contraction.
Rat models of SCI and DM demonstrate a marked difference in expression of bladder MR transcripts between the two disease states. This likely contributes to the divergent patterns of bladder activity in SCI and DM and suggests alternative pharmacologic strategies for treatment.

Concluding message

Our study suggests a new mechanism of action for anti-cholinergics in the treatment of OAB and may help explain the local effects of intravesical instillations of anti-cholinergics such as oxybutynin.