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MOLECULAR CHARACTERISATION OF MUSCARINIC RECEPTOR SUBTYPES IN PATIENTS WITH IDIOPATHIC DETRUSOR OVERACTIVITY (IDO)

Hypothesis / aims of study

Muscarinic receptor antagonists are widely used in treatment of patients with detrusor overactivity. Radioligand binding and molecular studies have shown that the human bladder detrusor expresses both M_2 and M_3 receptors (1). The majority of the receptor subtypes are M_2 (~70%), although detrusor contraction in response to parasympathetic nerve stimulation is mediated by M_3 receptors.

The aim of this study was to compare the expression of muscarinic receptor mRNA in detrusor of idiopathic detrusor overactivity (IDO) and control patients.

Study design, materials and methods

Biopsies of human detrusor were collected at cystoscopy from patients with IDO (n = 13) and age-matched control (n = 14) patients (age range 43-72 years), in accordance with the local hospital ethics approval. Control patients were undergoing cystoscopy due to a history of bladder cancer or to asymptomatic haematuria. All control patients displayed normal micturition frequency, with no urge incontinence.

IDO patients had urodynamically proven DO which was refractory to treatment, that is, patients failed to respond to more than two anticholinergic drugs and detailed bladder training for more than one year. Biopsies were taken from IDO patients in order to exclude carcinoma in situ or coexistent IC. Biopsies were collected into RNA later, dissected into detrusor muscle and mucosa and stored at $-70^{o}C$ until RNA extraction. Each QCRT-PCR analysis required 1.5 µg of RNA therefore each biopsy could not always be used to study both muscarinic receptor subtypes (M_2 or M_3), as RNA yield was limited (2 to 5 µg RNA/ biopsy).

The expression of M_2 and M_3 muscarinic receptors was quantified using quantitative competitive RT-PCR (QCRT-PCR) (1). Bladder RNA (100 ng/tube) was co-amplified with serial dilutions (30 – 0.03 pg/ tube) of competitor RNA, using RT-PCR. The products were then separated by gel electrophoresis (2.5% agarose) and quantified by densitometry. The amount of sample mRNA was determined by plotting the intensity of DNA bands versus the known concentration of competitor RNA.

Expression of β-actin mRNA was determined by RT-PCR and used as an internal control to monitor RNA quality. The QCRT-PCR data for M_2 and M_3 receptor expression in detrusor muscle were then normalised for the expression of β-actin in the same sample.

Results

A threefold increase in the median expression of M_3 receptor mRNA was seen in IDO patients (9.4 pg M_3 RNA/ 100ng bladder RNA; n = 13) compared with control (3.2 pg M_3 RNA/ 100ng bladder RNA, n = 14, P = 0.024, Mann Whitney test). No change in M_2 receptor mRNA expression was seen but sample size is small to date (n = 7). The target size is 20 for each clinical group and for each muscarinic subtype.

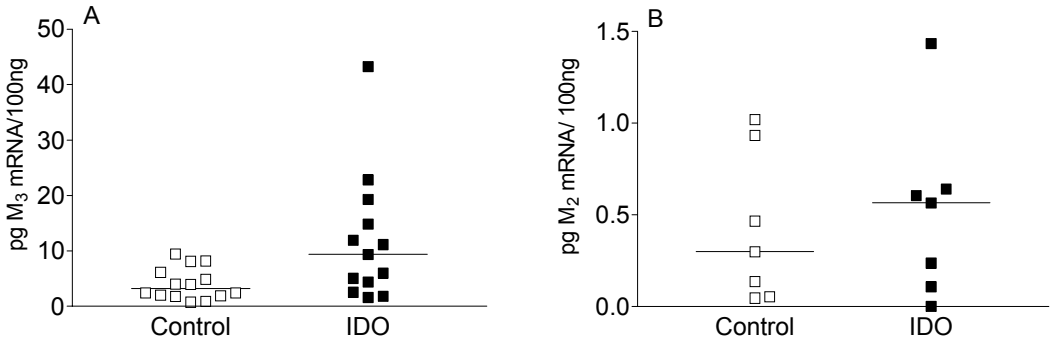

Figure 1. *Comparison of detrusor muscarinic receptor mRNA expression in control and idiopathic detrusor overactivity patients. (A), there was a significant increase in M_3 receptor mRNA expression in IDO detrusor (P = 0.024, n = 13). (B), M_2 receptor mRNA expression was not seen to change with disease (n = 7, P = 0.8).*

Interpretation of results

Results presented here demonstrate a significant increase in the expression of M_3 receptor mRNA in IDO patients. Functional studies have shown that activation of M_3 muscarinic receptors is primarily responsible for bladder contraction in response to parasympathetic nerve stimulation (2). Thus our present finding may indicate a role of M_3 receptors in the pathophysiology of IDO.

However, an alternative explanation should be considered. The IDO patients included in this study were refractory to >12 months anticholinergic therapy, and thus the increased expression of M_3 mRNA could possibly represent drug-induced upregulation of the target receptors.

Concluding message

There was a threefold increase in expression of M_3 receptor mRNA in the detrusor of IDO patients, compared to control. This is the first available report describing the expression of muscarinic receptors in human bladder from refractory detrusor overactivity patients.

References

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2. Autonomic & Autocoid Pharmacol (2000) 22: 133-145.

FUNDING: Australasian Urolological Foundation