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AGE-RELATED DECREASE IN MUSCARINIC M3 BUT NOT M2 RECEPTOR MRNA IN THE MALE DETRUSOR MUSCLE.

Hypothesis / aims of study

Urodynamic tests have demonstrated an age-related reduction in bladder capacity and increased incidence of detrusor overactivity as well as decreased flow rate and poor bladder emptying (1). In normal adults, bladder contraction is almost entirely due to activation of muscarinic receptors by acetylcholine released from parasympathetic nerves. Functional studies have shown that ageing is associated with a decrease in responsiveness of the detrusor to acetylcholine (2). Radioligand binding results have demonstrated a decrease in the density of muscarinic receptors with age (3) although which specific muscarinic receptor subtype(s) decrease with age has not been determined. The main aim of this study was to determine whether the expression of M_2 or M_3 muscarinic receptors was altered in ageing, in control patients. Our secondary aim was to determine whether the expression of β-actin was age- or gender-dependent, since this agent is used as an "internal control" in RT-PCR studies.

Study design, materials and methods

Biopsies of normal human detrusor were collected from 22 male and 22 female control patients (age range 18 – 88 years) undergoing cystoscopy due to a history of bladder cancer or to asymptomatic haematuria. All patients displayed normal micturition frequency, with no urge incontinence, and no apparent obstruction. Biopsies were taken from macroscopically normal areas of the bladder and were collected into RNA later, dissected into detrusor muscle and mucosa and stored at –70°C until RNA extraction. The expression of M_2 and M_3 receptor subtypes was quantified using quantitative competitive RT-PCR (QCRT-PCR). To produce competitor RNA for M_2 and M_3 muscarinic receptors, cDNA fragments (380 and 400 bp respectively) were reverse transcribed and amplified from human bladder mRNA by RT-PCR, and then internally deleted ~20% using restriction enzymes. The modified cDNAs were cloned into a vector and used as templates to synthesise competitor RNA. For QCRT-PCR, 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.

The expression of β-actin mRNA was determined by RT-PCR. 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

The expression of β-actin mRNA did not vary significantly with gender (Fig 1A) or with age (Fig 1B).

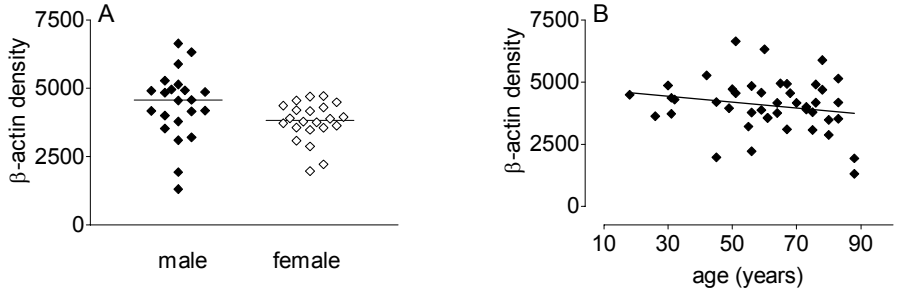

Figure 1. *Expression of β-actin mRNA in human bladder detrusor. (A) shows no significant difference in expression of β-actin in male and female (P = 0.06). (B), absence of any correlation of β-actin expression with age (r^2 = 0.038, P = 0.2, N = 44 males and females).*

In male patients, the expression of M_2 receptors remained relatively constant with age (Fig 2A) whereas the expression of M_3 receptors demonstrated a significant decrease with age (Fig 2B, P = 0.003). The mean expression of M_3 receptor mRNA was 3.6 ± 1.03 pg mRNA/100 ng RNA (N = 21), whereas that for M_2 receptor mRNA was 0.28 ± 0.07 pg mRNA/100 ng RNA (N = 16).

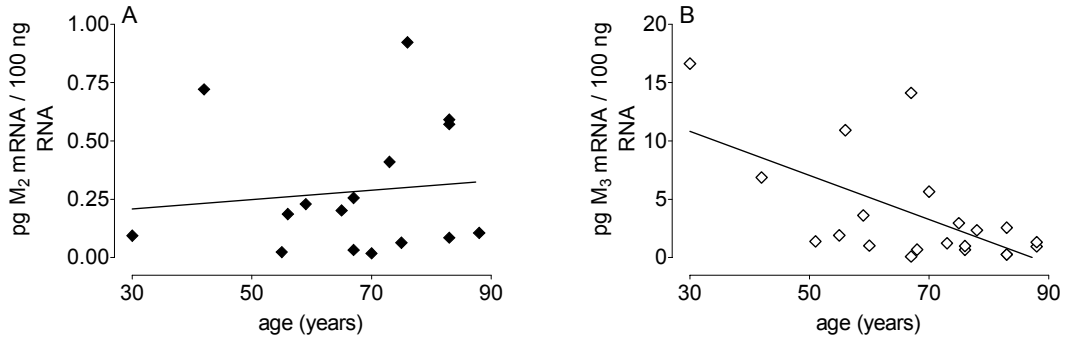

Figure 2. *Changes in muscarinic receptor mRNA expression with age, in male control patients. (A), M_2 receptor mRNA expression remained constant with age (r^2 = 0.013, P = 0.68, N = 16). (B), M_3 receptor mRNA expression decreased with age (r^2 = 38, P = 0.003, N = 21).*

Interpretation of results

In order to correct for any differences in RNA quality, QCRT-PCR results should be normalised to an internal control, and it is important that this does not vary with either gender or age. β-actin is widely used as an internal control and here we show that it is a suitable choice for studies in detrusor muscle. Although it appears that mRNA for M_3 receptors was greater than that of M_2 receptors, it is not valid to use QCRT-PCR to compare expression of different receptors since different primers and experimental conditions are involved in each case; moreover, mRNA expression is not necessarily linked to protein expression.

Previous radioligand binding studies using the muscarinic ligand [^{3}H]-QNB have demonstrated a decrease in the density (Bmax) of muscarinic receptors with age in the male detrusor (3). In that study, the subtype of muscarinic receptor decreasing with age could not be determined, although M_2 and M_3 receptors were the main muscarinic receptor types (70% M_2: 20% M_3). The present study has shown that it is the M_3 receptor expression which decreases with age in the male detrusor. Such a hypothesis would be in keeping with the known decrease in contractile responses to acetylcholine in the ageing detrusor (2).

In the isolated detrusor, contractile responses to acetylcholine are mediated by M_3 receptors. Clinical urodynamic studies in ageing patients have demonstrated several types of lower urinary tract dysfunction. Some of these features, such as decreased contractility and poor emptying, could be the direct result of the decrease in expression of M_3 receptor mRNA with age.

Concluding message

Radioligand binding studies have revealed an overall decrease in muscarinic receptor density (Bmax) in detrusor with increasing age. We have now shown a specific decrease in expression of M_3 but no change in M_2 muscarinic receptor mRNA with increasing age. This has not previously been reported.

References

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2. Exp Geront 36: 99-109, 2001.
3. Neurourol & Urodynamics 22: 527-529, 2003.

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