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MUSCARINIC RECEPTOR BINDING IN HUMAN DETRUSOR- AGE RELATED CHANGES.

Aims of Study

A costly consequence of old age is bladder dysfunction and incontinence. Ageing can bring disturbances in both the storage and voiding phases of bladder micturition. Urodynamic tests have demonstrated an age related reduction in bladder capacity, increased incidence of uninhibited contractions, decreased urinary flow rate, reduced voiding volume and incomplete bladder emptying (1, 2). Muscarinic receptor antagonists are the mainstay of therapy for patients with urinary incontinence disorders such as detrusor overactivity. However, such treatments are problematic due to side effects and a relative lack of antagonist subtype selectivity. The aim of this study was to use new subtype-selective muscarinic receptor antagonists and radioligand binding to investigate the affinity and receptor number of muscarinic receptors in detrusor, and to determine any age-related changes.

Methods

Specimens of normal human detrusor were collected from 21 control patients (median age 67, range 36–77 years; 15 males and 6 females) undergoing open bladder surgery (9 cystectomy, 9 radical prostatectomy, 3 colposuspension). Specimens were taken from macroscopically normal areas of the bladder and stored at –70^{o}C until use. All patients displayed normal micturition frequency, with no urge incontinence.

Radioligand binding with the muscarinic receptor ligand [^{3}H]quinuclidinyl benzylate (QNB) was performed on membranes from the detrusor muscle. After removal of the mucosa, membranes were prepared by homogenising the muscle in 50 mM sodium phosphate buffer (3). Incubations were carried out for 2 h at 37^{o}C. Nonspecific binding was defined using 10μM atropine. In saturation experiments, 8 concentrations of [^{3}H]QNB (15 pM to 2 nM) were incubated with membranes in 50 mM sodium phosphate buffer (pH 7.4). In competition experiments, 200 pM [^{3}H]QNB was incubated with increasing concentrations of a number of muscarinic receptor antagonists, whose subtype specificity is shown in Table 1. Incubations were terminated by filtration and washing with ice-cold buffer, through GF/B filters. Filter-bound radioactivity was quantified in a liquid scintillation counter. Protein content was determined by the Lowry method. Data were fitted to a one or two site model using the non-linear regression analysis program of GraphPad Prism (version 3) and are shown as mean pKi ± S.E.M. pKi is the negative log of the K_i.

Results

In saturation studies (n=12), [^{3}H]QNB bound to a single high affinity site, of dissociation constant (K_d) 55.7 ± 11.9 pM and maximum receptor number (Bmax) 152 ± 7.5 fmol/mg protein. Regression analysis demonstrated an inverse correlation of receptor number (Fig. 1A) and receptor affinity (Fig. 1B) with age (note that receptor affinity is inversely proportional to K_d). Further analysis revealed that the decrease in Bmax with age was due to a decline in M_2 receptor numbers, but no change in M_3 receptor numbers, with increasing age (Fig. 1C).

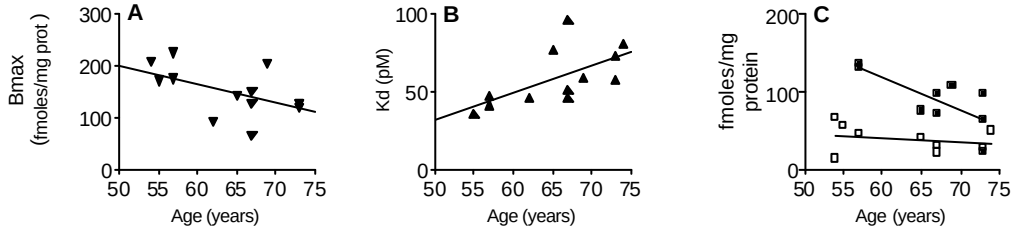

Figure 1. Regression analysis of [^{3}H]QNB saturation binding data for human detrusor muscle membranes. Panel A shows a decrease in receptor number (Bmax) with age (r^2 = 0.44, *P* = 0.019). In panel B the increasing Kd with age indicates a decrease in receptor affinity with

age (r^2 = 0.39, *P* = 0.03). Panel C demonstrates that the numbers of M_2 (■) receptors (r^2 = 0.55, *P* = 0.02) but not M_3 (□) receptors (r^2 = 0.07, *P* = 0.48) decline with increasing age.

In competition experiments, several new subtype selective muscarinic antagonists were investigated. Some competitors, 4-DAMP, darifenacin (M_3 receptor preferring) and methoctramine (M_2 receptor preferring), displayed biphasic binding profiles, with high and low affinity K_i values compatible with a population of approximately 75% M_2 receptors and 25% M_3 receptors (see Table 1). The mamba toxins MT1 (M_1 and M_4 receptor preferring) and MT3 (M_4 receptor preferring) showed poor affinity, consistent with negligible expression of these subtypes in detrusor muscle. However, pirenzepine (M_1 receptor preferring) showed some high affinity binding, possibly due to its affinity for M_3 receptors. There was no evidence for the presence of M_5 receptors.

Table 1. Radioligand binding data (mean ± S.E.M.) with [^{3}H]QNB in detrusor muscle membranes

Drug	Selectivity	pKi ± S.E.M.	pKi ± S.E.M. Site H*	pKi ± S.E.M. Site L*	% H
Atropine	nonselective	8.7 ± 0.6	N/A		
AQ-RA 741	M_2=M_3>M_5	8.1 ± 0.6	N/A		
4-DAMP	M_3>M_2	8.0 ± 0.6	9.7 ± 0.3	7.6 ± 0.5	22 ± 6
Methoctramine	M_2>M_3	7.5 ± 0.6	7.9 ± 0.6	6.2 ± 0.4	74 ± 5
Darifenacin	M_3>M_2	7.5 ± 0.6	9.7 ± 0.4	6.9 ± 0.6	23 ± 3
AF-DX 116	M_2>M_3	6.9 ± 0.5	N/A		
Pirenzepine	M_1>M_3>M_2	6.3 ± 0.6	8.9 ± 0.2	6.1 ± 0.6	15 ± 4
Mamba toxin 1	M_1=M_4	<<7.0	N/A		
Mamba toxin 3	M_4	<<7.0	N/A		

* data best fitted (P<0.05) to 2 site binding with high (H) and low (L) affinity components.
N/A: two site binding was not preferred, therefore only the one site binding results are shown

Conclusions

New subtype-selective muscarinic receptor antagonists have become available during the past decade. Previous studies examining muscarinic receptor binding in human detrusor were performed before these subtype-selective antagonists were available. This study concurs with previous findings that the M_2 subtype is the predominant muscarinic receptor in human detrusor. There is little evidence from our radioligand binding for the presence of M_1, M_4 or M_5 receptors in human detrusor. The novel finding from this work was the decrease in muscarinic receptor population with increasing age. This has not previously been reported, although functional detrusor muscle experiments have shown decreased contractile responses to acetylcholine with advancing age (4). The present study reveals that the age-related decline was specific for M_2 receptors, but not for M_3 receptors. These results raise the possibility of a role for M_2 receptors in the development of bladder contractility disorders, which are known to be more common in the elderly.

References

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