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COMPARISON OF THE EFFECTS OF MUSCARINIC RECEPTOR ANTAGONISTS ON CARBACHOL-INDUCED CONTRACTION IN HUMAN DETRUSOR BETWEEN NORMAL DETRUSOR AND DETRUSOR OVERACTIVITY

Hypothesis / aims of study

It has been reported that M3 muscarinic receptor subtypes mainly mediate contraction in normal bladder. It has also been reported that M_2-receptors participate in the contractile response under pathologic conditions in both rat and human detrusor smooth muscles. M2-receptors have been reported to mediate contraction in the rat with pelvic nerve denervation or spinal cord transaction, and in patients with neurogenic bladder dysfunction [1-3]. The aim of the present study is to compare the affinities of various muscarinic antagonists against carbachol-induced contraction in human detrusor muscles between normal state and detrusor overactivity.

Study design, materials and methods

Tissue samples of human bladder muscles were obtained from patients undergoing total cystectomy due to bladder cancer (normal bladder), and those undergoing prostatectomy due to benign prostatic hyperplasia or augmentation cystoplasty due to neurogenic bladder dysfunction (detrusor overactivity), and the mucosa and serosa were removed. The presence of detrusor overactivity was proven in video-urodynamic studies or ambulatory urodynamics before the surgery. Tissues were mounted in 5 ml organ baths containing Krebs solution, which was gassed with 95%O2 and 5% CO2. Resting tension of 1g was obtained. When the contraction had stabilized, increasing concentrations of carbachol, a muscarinic receptor agonist, were added cumulatively and concentration-response curves (CRCs) were obtained. After incubation for 30 minutes, a second CRC to carbachol was constructed in the continued presence of antagonist or vehicle. In this way, 4 CRCs to carbachol were obtained from the same strip, three in the presence of increasing concentrations of 4-DAMP (3,10,30nM), methoctramine (1,3 and 10μM), pirenzepine(1,3 and 10μM), trospium, tolterodine, propiverine, oxybutynin (3,10,30nM) or in the presence of vehicle. The correction factors of 1.0, 1.5 and 2.8 for EC_{50} values of the 2nd, 3rd and 4th CRCs, respectively, and 1.0, 0.85 and 0.67 for maximum responses of the 2nd, 3rd and 4th CRCs, respectively, were applied in all experiments. Antagonist affinities (PA_2 values) were determined by Schild regression when the slope of the Schild plot was not different from unity. When the antagonism was not competitive, antagonist dissociation constants (apparent K_B values) were calculated from the following equation: K_B= antagonist concentration (molar)/(dose ratio-1).The study has been conducted in accord with the Helsinki Declaration. The procedures have been approved by the local ethics committee, and written informed consent was obtained from each patient before entry into the study.

Results

Carbachol produced concentration-dependent contraction of the normal human urinary bladder with mean pEC_{50} values and maximum responses of 6.51±0.14 and 6.81±0.79 g, respectively. Carbachol produced concentration-dependent contraction of the human urinary bladder with detrusor overactivity with mean pEC50 values and maximum responses of 6.36±0.13 and 9.90±1.04 g, respectively. All muscarinic antagonists produced parallel, rightward displacement of the CRCs to carbachol without affecting maximum responses. Mean (±SEM) pA_2 (or apparent pK_B) values and Schild slopes for these antagonists were summarized in the table.

Interpretation of results

Excluding methoctramine for normal bladder and propiverine for normal and detrusor overactivity, all muscarinic receptor antagonists competitively antagonized CRCs to carbachol without changing maximum contractions and with Schild slopes not different from unity. 4-DAMP, a selective M3-receptor antagonist, oxybutynin, a selective M1- and M3- receptor antagonist, and trospium, tolterodine and propiverine, non-selective muscarinic receptor antagonists antagonized CRCs to carbachol with high affinities. Methoctramine, a M2-

receptor antagonist, and pirenzepine, a M1-receptor antagonist antagonized carbachol-induced contractions with relatively low affinities. These results suggest that M3-receptors mainly mediate contractions in normal human bladder. Antagonist affinities of muscarinic antagonists did not change in bladder strips with detrusor overactivity, indicating that M3-receptors still mediate contractions in this condition.

Table: Antagonist affinities of muscarinic antagonists against CRCs to carbachol

Antagonist	4DAMP	Methoctramine	Pirenzepine
Normal detrusor			
pA2(pKB)±SEM	9.87 0.09	6.64±0.05	7.38±0.09
Schild Slope	1.09±0.37	0.55±0.08*	0.77±0.17
Detrusor overactivity			
pA2(pKB)±SEM	9.01±0.12	6.18±0.09	7.33±0.11
Schild Slope	0.93±0.15	0.75±0.18	0.89±0.42

Antagonist	Trospium	Tolterodine	Propiverine	Oxybutynin
Normal detrusor				
pA2(pKB)±SEM	9.78±0.11	9.11±0.17	7.92±0.20	8.17±0.10
Schild Slope	0.91±0.29	1.10±0.08	0.56±0.60	0.89±0.12
Detrusor overactivity				
pA2(pKB)±SEM	9.71±0.64	8.70±0.16	8.30±0.11	8.39±0.92
Schild Slope	1.04±0.44	1.34±0.30	0.50±0.13*	0.99±0.14

Concluding message

Affinities of muscarinic antagonists did not change in patients with detrusor overactivity compared with those with normal state. M3-muscarinic receptor subtypes mainly mediate carbachol-induced contractions in human bladder with both normal state and detrusor overactivity.

References

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