529

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ANTIMUSCARINIC AGENTS BIND TO UROTHELIAL MUSCARINIC RECEPTORS WITH HIGH AFFINITY

Hypothesis / aims of study

The bladder urothelium not only provides a barrier to diffusion but also serves a sensory function and releases signaling molecules such as acetylcholine and ATP. Although the intrinsic ability of urothelium to make and release acetylcholine remains unclear, muscarinic receptors are present on the mucosa (urothelium) as well as detrusor muscle of the urinary bladder [1]. Therefore, mucosal muscarinic receptors may represent a novel site of action of antimuscarinic agents for the treatment of bladder disorders. In this connection, Kim et al. [2] showed that intravesically infused antimuscarinic agents suppressed carbachol-induced bladder overactivity, suggesting a blockage of muscarinic receptors in bladder-afferent pathways. However, the binding of antimuscarinic agents to urothelial muscarinic receptors have yet to be characterized. The aim of the present study was to characterize binding properties of several antimuscarinic agents to muscarinic receptors in the rat bladder urothelium.

Study design, materials and methods

The urothelium and detrusor were dissected from the rat bladder, and homogenized for the preparation of crude membranes. The muscarinic receptors in rat tissue homogenates were measured by a radioligand binding assay using [*N*-methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS), a selective radioligand of muscarinic receptors. For the quantitative determination of muscarinic receptor subtypes, the urothelium and detrusor of rats were treated with *N*-(2-chloroethyl)-4-piperidinyl diphenylacetate (4-DAMP mustard) as previously reported [3]. For experimental acute cystitis, rats received a single injection of cyclophosphamide (150 mg/kg, i. p.) and bladder muscarinic receptors were measured at 3 days after the injection.

Table 1 Inhibition constant (K_i values) and Hill coefficients (nH) for *in vitro* inhibition by imidafenacin, solifenacin, tolterodine, darifenacin, propiverine, DPr-P-4 (N→O) (active metabolite of propiverine) and oxybutynin of specific [^{3}H]NMS binding in the bladder urothelium and detrusor muscles of rats.

Antimuscarinics	Urothelium		Detrusor muscle	
	K_i (nM)	nH	K_i (nM)	nH
Imidafenacin	3.91 ± 0.84	1.10 ± 0.11	3.66 ± 0.46	1.04 ± 0.07
Solifenacin	64.3 ± 7.5	0.95 ± 0.15	56.4 ± 2.3	0.98 ± 0.01
Tolterodine	2.52 ± 0.18	1.07 ± 0.13	2.25 ± 0.25	1.02 ± 0.05
Darifenacin	17.4 ± 2.4	0.76 ± 0.09	18.0 ± 5.9	0.73 ± 0.06
Propiverine	328 ± 33	0.92 ± 0.05	348 ± 21	1.09 ± 0.17
DPr-P-4 (N→O)	186 ± 11	0.93 ± 0.07	231 ± 21	0.91 ± 0.04
Oxybutynin	11.2 ± 3.4	1.24 ± 0.09	7.09 ± 0.65*	1.19 ± 0.05

The binding of [^{3}H]NMS (0.5 nM) in rat tissues was measured in the absence and presence of different concentrations of imidafenacin, solifenacin, tolterodine, darifenacin, propiverine, DPr-P-4 (N→O) and oxybutynin. The K_i and Hill coefficients were estimated. Asterisks show a significant difference from values in the urothelium, *P<0.05. Each value represents the mean ± SEM for three to eight rats.

Results

Specific [^{3}H]NMS binding in the rat urothelium was saturable and of high affinity. The density and affinity of [^{3}H]NMS binding was not different between the urothelium and detrusor tissues. Moreover, antimuscarinic agents such as imidafenacin, solifenacin, tolterodine, darifenacin, propiverine, active metabolite of propiverine: DPr-P-4(N→O) was shown to bind to muscarinic receptors in the bladder urothelium with high affinity, and their binding affinities were quite similar to those in the detrusor muscle. Moreover, the pretreatment with 4-DAMP mustard, an irreversible inactivation agent of M_3 receptor subtype, caused significant reduction (36% and 22%, respectively) of [^{3}H]NMS binding sites in both urothelium and detrusor tissues of rats. Thus, the density of M_3 receptors was suggested to be higher in the urothelium than the detrusor muscle of rats. In the urothelium and detrusor muscle of cyclophosphamide-treated rats, the density of specific [^{3}H]NMS binding sites was significantly decreased compared with control rats.

Interpretation of results

The present study demonstrates that similar density of muscarinic receptors is present in the rat bladder urothelium and detrusor muscle. The density of M_3 receptors was suggested to be higher in the urothelium than the detrusor muscle of rats. The binding affinity of antimuscarinic agents to muscarinic receptors may be similar in the urothelium and detrusor of rats.

Concluding message

There are similar density of muscarinic receptors in the rat bladder urothelium and detrusor muscle. Antimuscarinic agents used currently to treat overactive bladder may bind to muscarinic receptors in the urothelium with high affinity, suggesting that these receptors are the pharmacological targets for antimuscarinic agents.

References

1. J Urol, 176: 1673-1678 (2006)

2. Urol, 65: 238-242 (2005)
3. J Pharmacol Exp Ther 313: 368-378 (2005)

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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