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AN ACTIVE METABOLITE (5-HYDROXYMETHYL TOLTERODINE: 5-HMT) OF FESOTERODINE EXCRETED IN THE URINE BINDS DIRECTLY TO MUSCARINIC RECEPTORS IN THE RAT BLADDER UROTHELIUM AND DETRUSOR MUSCLE

Hypothesis / aims of study

Fesoterodine is a relatively novel antimuscarinic agent for the treatment of the symptoms of overactive bladder [1]. When administered orally, fesoterodine is rapidly and extensively converted to its active metabolite, 5-hydroxymethyl tolterodine (5-HMT). Pharmacologically significant amount (16%) of 5-HMT is excreted in urine of human taking clinical dose (4, 8 mg) of fesoterodine [2]. Our current study with radioligand binding assay has shown that pharmacologically relevant muscarinic receptors are present in the bladder urothelium and detrusor muscles, and 5-HMT and other antimuscarinic agents bind these receptors with high affinity [3]. Thus, the excreted 5-HMT may be pharmacologically relevant under the *in vivo* condition after the oral administration of fesoterodine. In the current study, such hypothesis was investigated by measuring directly muscarinic receptor binding in the urothelium and detrusor muscles of rats after the intravesical instillation of 5-HMT.

Study design, materials and methods

The bladder tissues of rats were dissected in the urothelium and detrusor. Muscarinic receptors were measured by radioreceptor assay using [N-methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS), a selective radioligand of muscarinic receptors. Specific [^{3}H]NMS binding was measured in the presence of various concentrations of 5-HMT and other antimuscarinic agents. Furthermore, rats were intravesically instilled 5-HMT and other antimuscarinic agents for 30 min, and then muscarinic receptors in the urothelium and detrusor were simultaneously measured. Binding parameters of apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [^{3}H]NMS were estimated by nonlinear regression analysis using Graph Pad Prism. The inhibition constant, K_i, was calculated from the equation, $K_i=IC_{50}/(1+L/K_d)$, where IC_{50} and L represent the molar concentration of antimuscarinic agents necessary to displace 50 % of specific [^{3}H]NMS binding and the concentration of [^{3}H]NMS, respectively.

Results

5-HMT, tolterodine, oxybutynin and trospium inhibited concentration-dependently specific [^{3}H]NMS binding in the urothelium and detrusor muscle of rats, and their K_i values were little significantly different between these tissues (Table 1). 5-HMT displayed extremely high affinity to these bladder muscarinic receptors. Following the intravesical instillation of 5-HMT (300 nM, 3 □M), tolterodine (30, 300 nM) and oxybutynin (3 □M), there was a significant muscarinic receptor binding (increase in K_d for specific [^{3}H]NMS binding) in the bladder urothelium and detrusor of rats, compared with control values (Table 2). Interestingly, intravesical instillation of trospium (3-300 nM) showed little significant binding of muscarinic receptors in the urothelium and detrusor of rats.

Interpretation of result

5-HMT showed extremely high affinity to muscarinic receptors in the urothelium and detrusor muscle of rats, and the intravesically instilled 5-HMT bound significantly to these muscarinic receptors. Pharmacologically significant amount (16%) of 5-HMT is excreted in urine of human taking clinical dose (4, 8 mg) of fesoterodine [2]. Taken together, 5-HMT excreted in the urine, may bind significantly to bladder muscarinic receptors, thereby exerting significant effect on the bladder function. No significant muscarinic receptor binding of intravesical trospium may be attributable to the poor permeability due to the quaternary ammonium group in the chemical structure.

Concluding message

5-HMT binds muscarinic receptors not only in the detrusor muscle but also in the urothelium with high affinity. The function of these bladder muscarinic receptors may be significantly affected by 5-HMT excreted in the urine, thereby associating with the bladder selectivity of fesoterodine after the oral administration.

Table 1. K_i values for in vitro inhibition by 5-HMT and antimuscarinic agents of specific [^{3}H]NMS binding in the rats urothelium and detrusor muscle

Drug	Urothelium		Detrusor muscle	
	K_i (nM)	nH	K_i (nM)	nH
5-HMT	0.53 ± 0.02	0.91 ± 0.04	0.54 ± 0.03	0.96 ± 0.04
Tolterodine	2.52 ± 0.18	1.07 ± 0.13	2.25 ± 0.25	1.02 ± 0.05
Oxybutynin	11.2 ± 3.4	1.24 ± 0.09	7.09 ± 0.65*	1.19 ± 0.05
Trospium	0.82 ± 0.05	1.17 ± 0.06	0.83 ± 0.08	1.33 ± 0.13

Values are mean ± S.E. of 3 to 6 rats. *P<0.05 vs urothelium (unpaired t-test).

Table 2. K_d and B_{max} for specific [^{3}H]NMS binding in the urothelium and detrusor muscle of rats after intravesical instillation of 5-HMT and antimuscarinic agents for 0.5 hr

Drug		Urothelium		Detrusor muscle	
		K_d (pM)	B_{max} (fmol/mg protein)	K_d (pM)	B_{max} (fmol/mg protein)
Control		273 ± 5	107 ± 4	268 ± 6	118 ± 4
5-HMT	300 nM	350 ± 21* (1.28)	120 ± 10	321 ± 14* (1.20)	116 ± 11
	3 □M	414 ± 21** (1.52)	123 ± 12	368 ± 36** (1.37)	125 ± 19
Tolterodine	30 nM	348 ± 4** (1.27)	108 ± 14	333 ± 26** (1.24)	120 ± 16
	300 nM	319 ± 5** (1.17)	105 ± 10	329 ± 25** (1.23)	124 ± 11
Oxybutynin	3 □M	449 ± 35*** (1.64)	102 ± 7	349 ± 36*** (1.30)	106 ± 7
Trospium	3 nM	294 ± 24	102 ± 8	255 ± 33	104 ± 11
	30 nM	324 ± 27	116 ± 9	268 ± 28	114 ± 12
	300 nM	275 ± 37	117 ± 22	279 ± 15	110 ± 12

Values are means ± S.E. of 4-7 rats. Values in parentheses represent the fold-increase in K_d values relative to control. *P<0.05, **P<0.01, ***P<0.001 vs control values.

References
1. 1 Malhotra B et al., Curr Med Chem, 16: 4481 (2009)
2. 2 Ellsworth P et al., Ther Clin Risk Manag, 5: 869 (2009)
3. 3 Yoshida A et al., Urol (in press)

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