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Yoshida M[1], Masunaga K[2], Nagata T[3], Miyamoto Y[1], Haba T[1], Kudoh J[1]
***1.** Kumamoto Rosai Hospital, **2.** Tokyo Metropolitan Geriatric Hospital, **3.** Toshiba Hospital*

MUSCARINIC RECEPTORS-MEDIATED CONTROL OF NORADRENALIN RELEASE FROM ISOLATED HUMAN PROXIMAL URETHRAL SMOOTH MUSCLES

Hypothesis / aims of study

In the urethra, several reports suggested that muscarinic receptors on prejunctional site of adrenergic nerve ending might regulate noradrenalin releases from the nerves, resulting in the control of urethral tone (1). The present study was designed to compare the effects of various antimuscarinic drugs (pirenzepine, methoctoramine, 4-DAMP, oxybutynin, propiverine tolterodine and darifenacin) on noradrenalin release in human isolated proximal urethral smooth muscles, using microdialysis procedure.

Study design, materials and methods

Human proximal urethras were obtained from patients undergoing radical cysto-urethrectomy due to bladder carcinoma (mean age 68.4 years). Smooth muscle strips were dissected from the proximal urethra of each patient. Microdialysis probe was inserted into each strip, and Ringer solution (NaCl 147 mM, KCl 4 mM, $CaCl_2$ 2.3 mM; the pH was adjusted to 7.4 with NaOH) containing 0.05 mM ascorbic acid was perfused into the probe. Each strip with microdialysis probe was suspended in an organ bath filled with oxygenated Krebs-Henseleit solution. Each strip was connected to an isometric force displacement transducer, and an isometric tension development was recorded. The effects of pre-treatment with various antimuscarinic drugs on the contractile responses induced by electrical field stimulation (EFS) were evaluated. In addition, the dialysate during EFS (supramaximum voltage, 0.5 msec duration, 40 Hz and 3 sec train at 1 min interval for 10 min) was collected, and the amount of NA released in the dialysate was measured by HPLC with ECD, as previously described with only slight modification (2).

Results

EFS caused frequency-dependent contractile responses and noradrenalin releases in human isolated proximal urethral strips. Pre-treatment with pirenzepine (M1 receptors selective drug: 0.01-1.0 µM) and 4 DAMP (M3 receptors selective drug: 0.01-1.0 µM) did not cause significant changes in EFS-induced contractile responses and noradrenalin releases. Pre-treatment with methoctoramine (0.01 - 1.0 µM: M2 receptors selective drug) showed significant and concentration-depended increases in EFS-induced contractile response and noradrenalin release ($p<0.01$). Pre-treatment with propiverine, oxybutynin and darifenacin at the concentration up to 1.0 µM showed no significant effects on the EFS-induced contractile response and noradrenalin release. However, tolterodine (0.01-1.0 µM) caused concentration-dependent increases in EFS-induced contractile response and noradrenalin release in human proximal urethral smooth muscles (table 1).

Interpretation of results

Pre-treatment with methoctoramine significantly caused decrease in EFS-induced contractile response and noradrenalin release. The data suggested that M2 receptors in the prejunctional site of adrenergic nerve endings might be related to the inhibitory control of noradrenalin release from human proximal urethral smooth muscles. Among various antimuscarinic drugs clinically used, only tolterodine increased EFS-induced noradrenalin release and contractile response. Relatively higher affinity to M2 receptors of tolterodine may contribute to the significant inhibitory effect on M2 receptors of the prejunctional site of adrenergic nerve endings in human proximal urethral smooth muscles, resulting in increase in noradrenalin release. The present data suggest that tolterodine may cause increase in urethral tone, which may be related to the inhibitory action on incontinence.

Concluding message

The data showed that there were M2 receptors on prejunctional site of adrenergic nerve endings in human proximal urethral smooth muscles, which might have inhibitory control of noradrenalin release. Tolterodine may show inhibitory action on M2 receptors of prejunctional site, resulting in increase in the noradrenalin releases, and increase in the human proximal urethral tone.

Table 1. Effects of various antimuscarinic drugs (1.0 µM) on EFS-induced noradrenalin releases in human proximal urethral smooth muscles

Drugs	% of control values of noradrenalin releases
Pirenzepine (n=5)	87.6±4.5
Methoctoramine (n=5)	145.1±3.8*
4-DAMP (n=5)	105.1±3.3
Oxybutynin (n=5)	88.2±2.2
Propiverine (n=5)	95.0±5.18
Darifenacin (n=7)	92.9±2.4
Tolterodine (n=7)	128.6±4.0*

Value is represented as % of control value. Control values are EFS-induced noradrenalin releases without treatment with various drugs. * P < 0.05; significantly different from control values.

References

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2. J. Pharmacol. Exp. Ther. 255, 1090-1097, 1990

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Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**