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Original Article: Laboratory Investigation

Propiverine increases urethral wall catecholamine levels and bladder leak point pressure in rats

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Abbreviations & Acronyms
LPP = leak point pressure
SIF = small and intensely fluorescent

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Objective: To investigate whether propiverine has a noradrenaline re-uptake inhibitor and whether it acts on the lumbosacral cord or the urethral wall. In addition, we aimed to examine the effect of propiverine on leak point pressure in rats.

Methods: A total of 72 female and 30 male rats were used to examine the following: (i) the change of leak point pressure caused by intravenous agents in rats with vaginal distention; (ii) the change of leak point pressure caused by intrathecal agents in rats with vaginal distention; (iii) the noradrenaline re-uptake inhibitor action of propiverine; and (iv) catecholamine levels in the bladder wall, urethral wall, cerebrospinal fluid and plasma after oral administration of propiverine.

Results: Intravenous injection of propiverine, imipramine and duloxetine increased the leak point pressure in rats with vaginal distention. Intrathecal naftopidil decreased the leak point pressure, whereas subsequent intravenous propiverine restored the leak point pressure to the level before intrathecal naftopidil in rats with vaginal distention. Propiverine acted like a noradrenaline re-uptake inhibitor, increasing noradrenaline and/or dopamine levels in the plasma, cerebrospinal fluid, and urethral wall perfusion fluid.

Conclusion: Propiverine inhibits noradrenaline re-uptake, as well as having antimuscarinic and Ca-antagonist actions. The inhibition of noradrenaline re-uptake by propiverine mainly occurs at the urethral level and partially in the central nervous system, and might stimulate the smooth muscle of the bladder neck and proximal urethra through α1-adrenergic receptors, as well as stimulating the striated muscle of the urethra and pelvic floor by activation of spinal motoneurons. Therefore, propiverine might be effective for both stress and urge incontinence.

Key words: catecholamine, leak point pressure, propiverine, re-uptake inhibitor, stress urinary incontinence.

Introduction

Stress incontinence is a urinary symptom that has a marked influence on the quality of life of women from their 20s to those who are middle-aged or elderly.[1] Kegel exercises, bladder training and surgery are mainly used for the treatment of stress incontinence, with few medications being indicated for this condition. In Europe, the antidepressant duloxetine hydrochloride (duloxetine) is used to treat stress incontinence.[2,3] In Japan, clenbuterol hydrochloride,[4] a β-agonist that is used to treat asthma, is the only drug indicated for stress incontinence, although the tricyclic antidepressant imipramine hydrochloride (imipramine) is also employed for off-label use.[2,5] Propiverine hydrochloride (propiverine) is an anticholinergic and Ca antagonist that is indicated for urge incontinence, but is administered to patients with mixed urge and stress incontinence in daily practice. It has generally been found that both types of incontinence are improved by propiverine treatment, and some reports about the efficacy of propiverine for pure stress incontinence have also been published in Japan.[6] Anticholinergic drugs reduce bladder hypersensitivity, and thus are indicated for patients with urge incontinence as a result of involuntary bladder contractions (overactive bladder). However, the concept that anticholinergic drugs are ineffective because stress incontinence is due to a low urethral closing pressure has resulted in doctors not prescribing these drugs for patients with stress incontinence.

A recent animal study of the anticholinergic agent propiverine showed that blood catecholamine levels and the urethral closing pressure were elevated by administration of this drug for 2 weeks to young rats.[7–9] Noradrenergic sympathetic pathways mediate excitatory inputs to the bladder neck and the proximal urethra through α1-adrenergic receptors,[10,11] as well as inhibitory inputs to the body of the bladder through β2- or β3-adrenergic receptors.[12,13] Therefore, we recently re-examined the effect of propiverine (20–40 mg/day) on stress incontinence and mixed incontinence in women, and confirmed that this drug improved both types of incontinence by increasing the maximum urethral closing pressure and the functional urethral length.[14] However, the mechanism through which propiverine acts to improve urethral function is still unknown.

Therefore, we investigated whether propiverine has a noradrenaline re-uptake inhibitor effect like imipramine or duloxetine,[15] and whether propiverine acts at the level of the lumbosacral cord or the urethral wall. We also examined whether the effect of propiverine on LPP was equivalent to that of imipramine or duloxetine in rats.

Methods

Materials

A total of 72 female and 30 male Sprague–Dawley rats (7–9 weeks old) weighing 200–245 g were used. The protocol for the present study was approved by the President of University of the Ryukyus based on the judgment of our Institutional Animal Care and Use Committee.

Experiment 1: Measurement of LPP before and after intravenous injection of imidafenacin, propiverine, imipramine and duloxetine in rats with vaginal distention

In 32 female rats, a modified 10-Fr Foley balloon catheter with the tip cut off was inserted into the vagina under 2% isoflurane anesthesia, and the vaginal orifice was closed with a suture to prevent the catheter from coming out. Then the balloon was inflated with 4 mL of water to distend the vagina for 3 h, after which the catheter was removed.[16] Four days later, a lower abdominal incision was made under urethane anesthesia (0.3 g/kg intraperitoneally and 0.9 g/kg subcutaneously), and a polyethylene catheter (PE-50; Clay Adams, Franklin Lakes, NJ, USA) was inserted through the dome of the bladder and connected to a pressure transducer to record the intravesical pressure. The visceral branches of the pelvic nerves were transected bilaterally near the internal iliac vessels to abolish reflex bladder contractions, and the abdominal incision was closed with sutures. A cannula was inserted into a femoral vein for intravenous injection of drugs. Bilateral incisions were made near the tips of the 11th–13th ribs to expose the oblique abdominal muscles for electrical stimulation, and the animal was placed in the supine position for the experiment.[17] After the bladder was emptied, 0.3 mL of saline containing Evans blue (100 μg/mL) was injected. While the intravesical pressure was recorded, the exposed abdominal muscles were stimulated with an electrical stimulator (SEN-7203; Nihon Kohden, Tokyo, Japan) and an isolator (SS-202J; Nihon Kohden). Electrical stimulation (rectangular pulses with a duration of 0.5 ms at 20 ms intervals for 1 s) was repeated every 10 s, and the stimulus intensity was gradually increased over the range between 2 and 25 V to increase the intravesical pressure in a stepwise manner. The pressure at which fluid leaked from the urethral orifice was defined as the LPP.[9] The LPP was measured before and 3 min after intravenous injection (cumulative doses: 0.1, 0.3, 1 and 3 mg/kg) of imidafenacin (an antimuscarinic agent), propiverine, imipramine or duloxetine. Imidafenacin, which is used for the treatment of overactive bladder, was dissolved in 0.2–0.6 mL of 1% acetic acid, while propiverine, imipramine and duloxetine were dissolved in 0.2–0.6 mL of physiological saline.[18]

Experiment 2: Measurement of LPP before and after intrathecal injection of naftopidil and intravenous injection of propiverine in rats with vaginal distention

Four days after vaginal distension, eight female rats were placed under urethane anesthesia and a fine polyethylene catheter (PE-10; Clay Adams) was inserted into the subarachnoid space at the level of lumbosacral cord (L6-S1) after L3 laminectomy for intrathecal injection of naftopidil. The LPP was measured as described for Experiment 1. Then naftopidil (30 μg/10 μL) was injected intrathecally and the LPP was measured again 2 min later. Naftopidil is an α-1D/A receptor antagonist used to treat benign prostate hyperplasia.[19] It was dissolved in 0.1 mol/L phosphate solution and injected intrathecally at a dose of 30 μg, which was sufficient to inhibit isovolumetric rhythmic bladder contraction for approximately 30 min.[19] At 10 min after intrathecal injection of naftopidil, propiverine (3 mg/kg) was injected intravenously and the LPP was measured.

Experiment 3: Assessment of the noradrenaline re-uptake inhibitor effect

In 30 male rats, the striatum was removed from rats after decapitation, weighed and homogenized in a 10-fold volume of 0.32 mol/L sucrose in an ice bath. The homogenate was centrifuged at 1000 *g* for 10 min, and the resulting supernatant was centrifuged at 17 000 *g* for 60 min. The membrane fraction thus obtained was washed with buffer by the same centrifugation procedure (200 μg tissue eq./mL). The noradrenaline hydrochloride, DL-[7-3H(N)] binding assay was carried out.[20] Briefly, nisoxetine or propiverine was incubated with rat striatal membranes (50 μL), 177.51 nmol/L noradrenaline hydrochloride, DL-[7-3H(N)] (100 μL) and buffer at 37°C for 5 min. After incubation, the mixture was filtered through a GF/C filter (Whatman; GE Healthcare UK Ltd, Buckinghamshire, UK), and then the filter was mixed with liquid scintillator (5 mL). The filters were rinsed three times with the same buffer. Radioactivity retained on the filters was measured by liquid scintillation (Tri-Carb 1500 and 3100; PerkinElmer, Waltham, MA, USA). Non-specific binding was defined as the binding in the presence of nisoxetine (1×10^{-5} mol/L).

Experiment 4: Measurement of catecholamines in the bladder wall, urethral wall, cerebrospinal fluid and plasma after oral administration of propiverine

A total of 32 female rats were divided into a control group (n = 16) and a propiverine group (n = 16). Rats in these groups were, respectively, treated once daily with 1 mL of distilled water or propiverine dissolved in distilled water (5 mg/mL) by gavage using a fine catheter.[8] Treatment was continued for 10 days to obtain reliable data, because the catecholamine level of the urethral perfusion fluid was relatively stable by 10 days of propiverine administration in the preliminary study Then, the animals were anesthetized with urethane and the following experiments were carried out.

In eight rats from each group, a lower abdominal incision was made. The bladder was emptied and the bladder neck was ligated. A disposable plastic indwelling cannula with an outer needle (24-G) and an inner needle (27-G; SR-OT2419C; Terumo Corporation, Tokyo, Japan) was inserted into the right side of the bladder. The inner needle was removed, inserted into the left side of the bladder and connected to a pump for infusion of physiological saline (2 mL/h). Then, bladder perfusion fluid was collected from the outer needle for approximately 1 h. In addition, blood was collected from the inferior vena cava and plasma was obtained. Cerebrospinal fluid was also collected by laminectomy at L3. In the remaining eight rats from each group, a lower abdominal incision was made. The bladder was emptied and the bladder neck was ligated. A disposable plastic indwelling cannula with outer and inner needles was inserted into the right wall of the urethra, and urethral perfusion fluid was collected from the outer needle in the same procedure as bladder. The samples of bladder perfusion fluid, urethral perfusion fluid, plasma and cerebrospinal fluid were immediately placed in a freezer at −30°C, and catecholamine levels were measured by BML Inc. (Tokyo, Japan).

Results are reported as the mean ± standard deviation. Statistical analyses were carried out by using the paired t-test, unpaired t-test, or one-way and two-way ANOVA followed by Tukey's test. A P-value <0.05 was considered to show statistical significance.

Results

Experiment 1

Electrical stimulation of the abdominal wall muscles with gradual elevation of the voltage increased the intravesical pressure in a stimulus-dependent manner, and leakage from the urethral orifice was detected during stimulation. When imidafenacin (0.1–3 mg/kg) was injected intravenously, the LPP did not change. However, injection of propiverine (3 mg/kg) increased the LPP compared with that before injection (P = 0.009; Fig. 1). Injection of imipramine (1 and 3 mg/kg) also (P = 0.032 and 0.009, respectively) increased the LPP, respectively, compared with that before injection. Furthermore, injection of duloxetine (0.1–3 mg/kg) increased the LPP (at 3 mg/kg) compared with that before injection (P = 0.001 at 3 mg/kg). There were no differences in the changes of LPP after intravenous injection of propiverine or imipramine compared with after intravenous duloxetine (Fig. 1).

Experiment 2

Baseline LPP was 26.2 ± 1.9 cmH_2O before intrathecal injection of naftopidil. Intrathecal injection of naftopidil decreased the LPP (P = 0.010; Fig. 2). After that, intravenous injection of propiverine (3 mg/kg) increased the LPP (P = 0.025) compared with that after intrathecal injection of naftopidil, but not compared with that before intrathecal naftopidil. After both intrathecal naftopidil and intravenous propiverine, the LPP was lower (P = 0.016) compared with that (44.9 ± 4.9 cmH_2O) after intravenous injection of 3 mg/kg of propiverine during Experiment 1.

Experiment 3

Effects of propiverine and nisoxetine on monoamine uptake by rat cerebral cortical synaptosomes. Rat striatal synaptosomes were used to assess uptake of the [^{3}H]ligand DL-[7-^{3}H

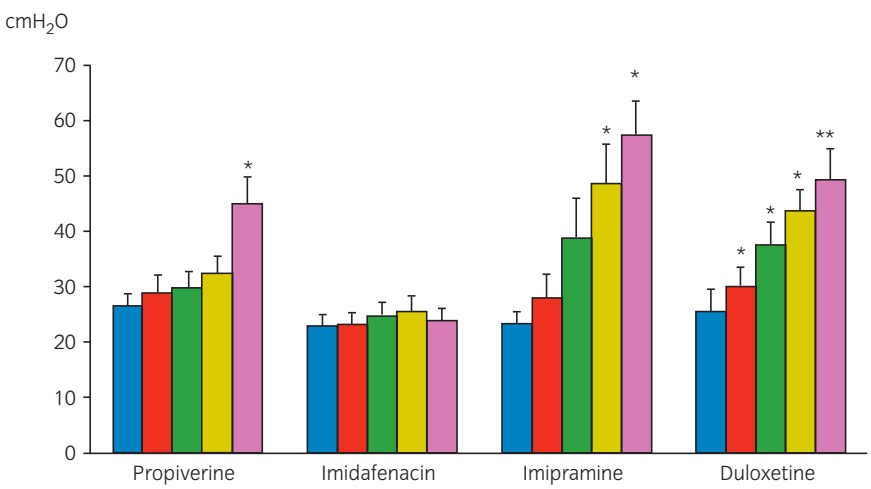

Fig. 1 Comparison of the LPP during electrical stimulation of the abdominal wall muscles after intravenous injection of test agents. After imidafenacin was injected intravenously, the LPP did not change. In contrast, intravenous injection of propiverine (3 mg/kg) significantly increased the LPP compared with before injection. Injection of imipramine and injection of duloxetine also significantly increased the LPP compared with before injection. Mean ± SE, *P < 0.05, **P < 0.01. (■) Before, (■) 0.1 mg/kg, (■) 0.3 mg/kg, (■) 1 mg/kg, (■) 3 mg/kg.

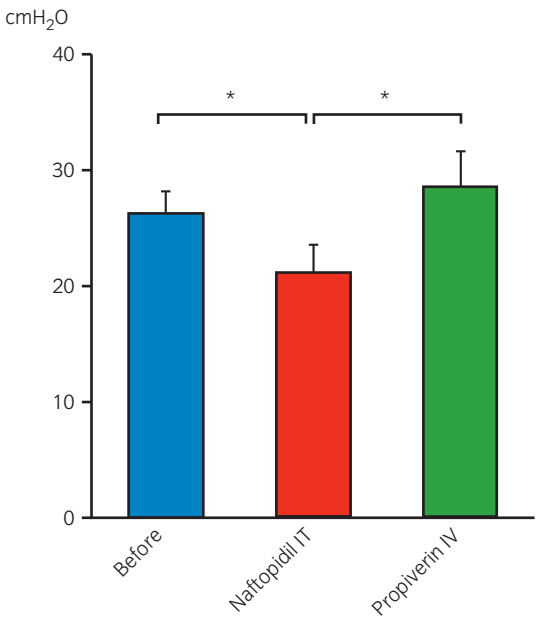

Fig. 2 Change of LPP after intravenous injection of propiverine with or without the α-1A/D blocker naftopidil. Intrathecal injection of naftopidil significantly decreased the LPP. Intravenous injection of propiverine (3 mg/kg) just after intrathecal naftopidil significantly restored the LPP compared with that after intrathecal naftopidil alone. Mean ± SE, *$P < 0.05$.

(N)] at 37°C for 5 min. Both propiverine and nisoxetine were shown to be potent inhibitors of noradrenaline uptake (the IC_{50} for propiverine was 1.69×10^{-5} mol/L and that for nisoxetine was 4.11×10^{-9} mol/L; Table 1).

Experiment 4

Plasma levels of noradrenaline and dopamine were higher in the propiverine group ($P = 0.001$ and <0.001, respectively) than in the control group (Fig. 3). In contrast, there was no difference of the plasma adrenaline level between the two groups. In the propiverine group, the noradrenaline level in the cerebrospinal fluid was higher ($P = 0.014$) than in the control group. Both adrenaline and dopamine were too low to be detected in the cerebrospinal fluid. In the propiverine group, the levels of noradrenaline and dopamine in the urethral wall perfusion fluid were higher ($P = 0.048$ and 0.033) than in the control group. In contrast, there was no difference of the adrenaline level in urethral wall perfusion fluid between the two groups. The levels of adrenaline, noradrenaline and dopamine in the bladder wall perfusion fluid of the propiverine group did not differ from those in the control group (Fig. 3).

Discussion

The levels of noradrenaline and dopamine in the lower urinary tract wall were approximately 10-fold and >100-fold higher than in the plasma, respectively. Although inhibition of dopamine re-uptake by propiverine has not been examined, these results suggested that propiverine also has a noradrenaline- and dopamine-re-uptake inhibitory effect. Propiver-

Table 1 Norepinephrine re-uptake inhibition ratio and IC50

Substance	Substance concentration (mol/L)											IC50 (mol/L)
	3×10^{-10}	1×10^{-9}	3×10^{-9}	1×10^{-8}	3×10^{-8}	1×10^{-7}	3×10^{-7}	1×10^{-6}	3×10^{-6}	1×10^{-5}	3×10^{-5}	1×10^{-4}	
Propiverine						0.39	2.89	6.33	10.74	37.46	67.41	86.05	1.69×10^{-5}
Nisoxetine	10.23	23.86	47.52	70.68	80.51	92.49							4.11×10^{-9}

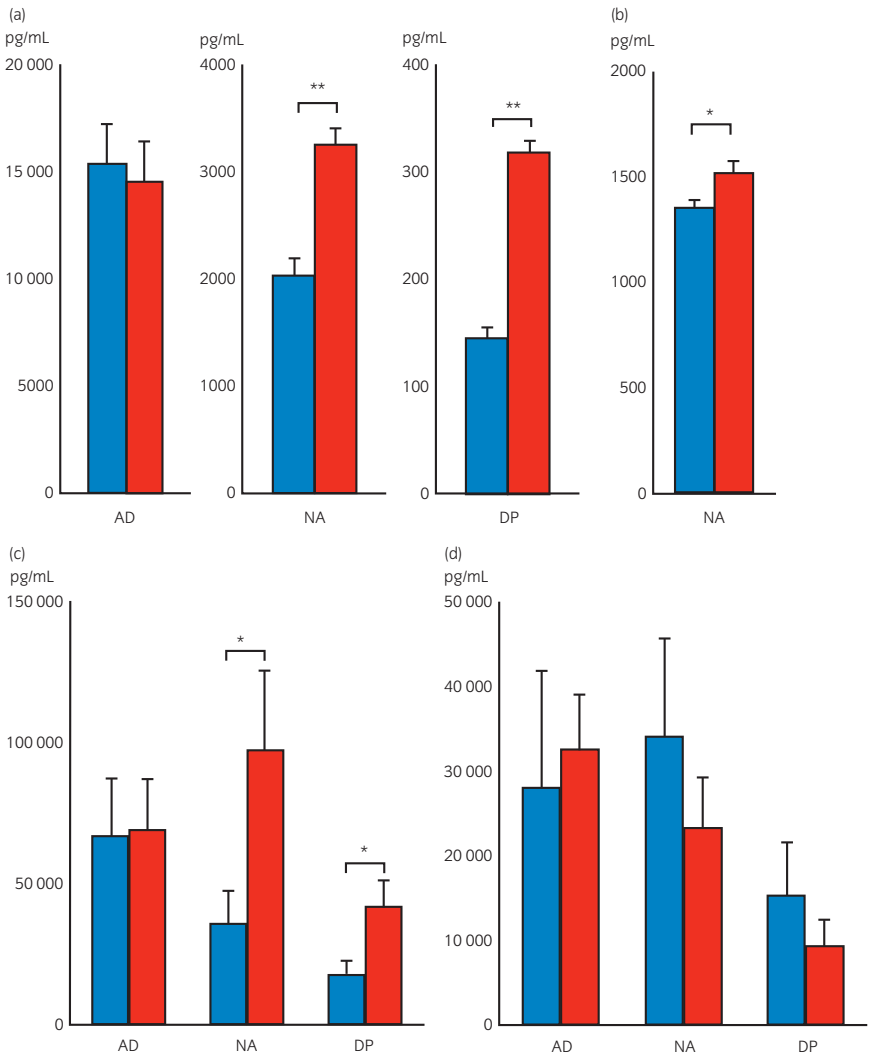

Fig. 3 Comparison of cathecholoamine levels in (a) the plasma, (b) cerebrospinal fluid, (c) urethral wall perfusion fluid and (d) bladder wall perfusion fluid. (a) Plasma noradrenaline and dopamine levels were significantly higher in the propiverine group than in the control group. (b) The cerebrospinal fluid noradrenaline level was significantly higher in the propiverine group than in the control group. Adrenaline and dopamine were too low to be detected in the cerebrospinal fluid. (c) Noradrenaline and dopamine levels in the urethral wall perfusion fluid were significantly higher in the propiverine group than in the control group. (d) There was no significant difference of the catecholamine levels in bladder wall perfusion fluid between the two groups. Mean ± SE, $*P < 0.05$, $**P < 0.01$. (■) Propiverine group, (■) control group.

ine increased the urethral levels of noradrenaline and dopamine by approximately 2- to 2.7-fold, and it also increased the plasma noradrenaline and dopamine levels by 1.6- to 2.2-fold. Therefore, plasma catecholamine levels might be involved by leakage of catecholamines from the peripheral organs in addition to from the adrenal glands.

Peripheral catecholamine-producing cells are closely associated with the sympathetic nervous system, and are often innervated by preganglionic sympathetic nerve fibers.[21] A number of sympathetic ganglia contain cells that are SIF, and these cells show tyrosine hydroxylase immunoreactivity.[22] Two types of SIF cells have been identified, which are interneurons (type I) and endocrine-like cells (type II).[23–25] The role of SIF cells in ganglionic systems is unknown, but it has been suggested that these cells might have an endocrine function (type II) or might modulate the synaptic output of neurons (intramural or major pelvic ganglions; type I).[23,24] In the rat lower urinary tract, tyrosine hydroxylase immunoreactivity SIF cells are mainly located in the bladder neck and proximal urethra, whereas fewer cells are found in the bladder.[26] Therefore, inhibition of noradrenaline re-uptake by propiverine might occur through an action on SIF cells and sympathetic postsynaptic terminals, especially in the urethra.

Our previous study showed that propiverine increased the baseline urethral pressure, with this effect on urethral pressure being lessened by intravenous administration of α-1 receptor antagonists, such as prazosin, silodosin or naftopidil.[9] The elevation of baseline urethral pressure might have been as a result of an excitatory effect of propiverine on smooth muscle in the bladder neck and proximal urethra, and/or an influence on the striated muscle of the external urethral sphincter. Noradrenaline is the neurotransmitter that mediates excitatory sympathetic inputs to the bladder neck and proximal urethra through α1A- and α1D-adrenergic receptors.[10,11] Dense noradrenaline-containing terminals and urethral rhabdosphincter motor neurons are located in the motor nucleus of the urethral sphincter (Onuf's nucleus) in the sacral spinal cord.[27] Therefore, an increase of catecholamines induced by administration of propiverine might activate both sympathetic pathways and Onuf's nucleus through α1A- and α1D-adrenergic receptors to increase the baseline urethral pressure.

Imipramine and duloxetine both act as noradrenaline and serotonin re-uptake inhibitors, with these actions at the lumbar spinal cord level increasing the urethral closing pressure.[9,28] In the present study, propiverine inhibited nora-

drenaline re-uptake and raised the noradrenaline level in the cerebrospinal fluid by 12%. Propiverine, imipramine and duloxetine all increased the LPP in rats with vaginal distension, also showing that these drugs increased urethral tone. Rats with vaginal distension are an animal model of stress incontinence as a result of simulated birth trauma.[16] In this model, intravenous injection of a noradrenaline re-uptake inhibitor (nisoxetine) increases the LPP, whereas this effect is reversed by intrathecal injection of α1-adrenergic receptor antagonists, such as phentolamine (a non-specific antagonist) and prazosin (a selective α1-receptor antagonist).[29] In the present study, intrathecal injection of the α-1D/A receptor antagonist naftopidil inhibited the effect of intravenous propiverine on the LPP. Active urethral closure in response to stress is mediated by activation of somatic nerves innervating the striated muscles of the urethra and pelvic floor.[30] Therefore, part of the mechanism by which propiverine is effective for stress incontinence might involve activation of spinal motoneurons innervating the urethral and pelvic floor striated muscle, because propiverine inhibits noradrenaline re-uptake and increases catecholamine levels.

It has been reported that plasma catecholamine levels increase with age. Administration of propiverine increased catecholamine levels in young rats, but did not further increase the elevated catecholamine levels in elderly rats.[8] Clinically, propiverine is effective for both stress and urge incontinence by increasing the maximum urethral closing pressure and functional urethral length without elevating the blood pressure, pulse rate or plasma catecholamine levels.[22] Therefore, we guess that propiverine administration can also increase the catecholamine level in urethral wall in the elderly people, but its level seems not to influence the plasma catecholamine level. It is already known that propiverine has antimuscarinic and Ca-antagonist actions, and that it inhibits bladder overactivity. The present study showed that propiverine also inhibits noradrenaline re-uptake (mainly in the urethra and partially in the central nervous system), and that these multiple actions of propiverine could be useful for the treatment of stress incontinence. However, these possibilities are speculation that does not exceed the imagination, and there is a limit to confirm at this time.

In conclusion, propiverine inhibits noradrenaline re-uptake, as well as having antimuscarinic and Ca-antagonist actions. Its noradrenaline re-uptake inhibitor effect is mainly exerted on the urethra and partially on the central nervous system, and might stimulate the smooth muscle of the bladder neck and proximal urethra through α1-adrenergic receptors, as well as the striated muscle of the urethra and pelvic floor by activation of spinal motoneurons. Therefore, propiverine might be effective for both stress and urge incontinence.

Acknowledgment

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Conflict of interest

None declared.

References

1 Homma Y, Yamaguchi O, Hayashi K; Neurogenic Bladder Society Committee. Epidermiologic survey on lower urinary tract symptoms in Japan. *Urology* 2006; **68**: 560–4.
2 Zinner NR, Koke SC, Viktrup L. Pharmacotherapy for stress urinary incontinence: present and future options. *Drugs* 2004; **64**: 1503–16.
3 Bump RC, Voss S, Beardsworth A *et al.* Long-term efficacy of duloxetine in women with stress urinary incontinence. *BJU Int.* 2008; **102**: 214–8.
4 Ushiroyama T, Ikeda A, Ueki M. Clinical efficacy of clenbuterol and propiverine in menopausal women with urinary incontinence: improvement in quality of life. *J. Med.* 2000; **31**: 311–9.
5 Lin HH, Sheu BC, Lo MC *et al.* Comparison of treatment outcomes for imipramine for female genuine stress incontinence. *Br. J. Obstet. Gynaecol.* 1999; **106**: 1089–92.
6 Watanabe H, Uchida M, Kojima M. Clinical effect of propiverine hydrochloride on urinary incontinence. *Hinyoukika Kiyo* 1998; **44**: 199–206.
7 Nishijima S, Sugaya K, Kadekawa K *et al.* Comparison of the effect of antimuscarinic agents on bladder activity, urinary ATP level, and autonomic nervous system in rats. *Biomed. Res.* 2009; **30**: 107–12.
8 Nishijima S, Sugaya K, Kadekawa K *et al.* Efficacy of propiverine, an anticholinergic agent, in young and old rats. *Life Sci.* 2011; **26**: 456–9.
9 Kadekawa K, Nishijima S, Ashitomi K *et al.* Excitatory effect of propiverine hydrochloride on urethral activity in rats. *Int. J. Urol.* 2012; **19**: 575–82.
10 Anderson KE, Gratzke C. Pharmacology of alpha1-adrenocveptor antagonists in the lower urinary tract and central nervous system. *Nat. Clin. Pract. Urol.* 2007; **4**: 368–78.
11 Sugaya K, Nishijima S, Miyazato M *et al.* Central nervous control of micturition and urine storage. *J. Smooth Muscle Res.* 2005; **41**: 117–32.
12 Igawa Y, Yamazaki Y, Takeda H *et al.* Functional and molecular biological evidence for a possible beta3-adrenoceptor in the human detrusor muscle. *Br. J. Pharmacol.* 1999; **126**: 819–25.
13 Wuest M, Eichhorn B, Grimm MO *et al.* Catecholamines relax detrusor through beta 2-adrenoceptors in mouse and beta 3-adrenoceptors in man. *J. Pharmacol. Exp. Ther.* 2009; **328**: 213–22.
14 Sugaya K, Sekiguchi Y, Satoh T *et al.* Effect of propiverine hydrochloride on stress urinary incontinence. *Int. J. Urol.* 2014; **21**: 1022–5.
15 Alberti C. Coadministration of low-dose serotonin/noradrenaline re-uptake inhibitor (SNRI) duloxetine with α 2-adrenoceptor blockers to treat both female and male mild-to-moderate stress urinary incontinence (SUI). *G. Chir.* 2013; **34**: 189–94.
16 Kamo I, Kaiho Y, Cannon T *et al.* Functional analysis of active urethral closure mechanism under sneeze-induced stress condition in a rat model of birth trauma. *J. Urol.* 2006; **176**: 2711–5.
17 Kamo I, Hashimoto T. Involvement of reflex urethral closure mechanisms in urethral resistance under momentary stress condition induced by electrical stimulation of rat abdomen. *Am. J. Physiol. Renal. Physiol.* 2007; **293**: 920–6.
18 Yamada S, Seki M, Ogoda M *et al.* Selective binding of bladder muscarinic receptors in relation to the pharmacokinetics of a novel antimuscarinic agent, imidafenacin, to treat overactive bladder. *J. Pharmacol. Exp. Ther.* 2011; **336**: 365–71.
19 Sugaya K, Nishijima S, Miyazato M *et al.* Effects of intrathecal injection of tamsulosin and naftopidil, alpha-1A and -1D adrenergic receptor antagonists, on bladder activity in rats. *Neurosci. Lett.* 2002; **328**: 74–6.
20 Lewin AH, de Costa BR, Rice KC *et al.* Meta- and para-isothiocyanato-t-butylbicycloorthobenzoate: irreversible ligands of the gamma-aminobutyric acid-regulated chloride ionophore. *Mol. Pharmacol.* 1989; **35**: 189–94.
21 Coupland RE, Bustami F. Influence of drugs affecting the pituitary adrenal axis on chromaffin cells. *Adv. Biochem. Psychopharmacol.* 1980; **25**: 175–83.
22 Doupe AJ, Patterson PH, Landis SC. Small intensely fluorescent cells in culture: role of glucocorticoids and growth factors in their development and interconversions with other neural crest derivatives. *J. Neurosci.* 1985; **5**: 2143–60.
23 Coupland RE. The natural history of the chromaffin cell–twenty-five years on the beginning. *Arch. Histol. Cytol.* 1989; **52**: 331–41.
24 Langley K, Grant NJ. Molecular markers of sympathoadrenal cells. *Cell Tissue Res.* 1999; **298**: 185–206.
25 Williams TH, Chiba T, Black AC *et al.* (eds). *Species variation in SIF cells of superior cervical ganglia: are there two functional types?*, 1st edn. U.S. Government Printing Office, Washington, DC, 1976.

26 Zvarova K, Vizzard MA. Distribution and fate of cocaine- and amphetamine-regulated transcript peptide (CARTp)-expressing cells in rat urinary bladder: a developmental study. *J. Comp. Neurol.* 2005; **5**: 501–17.
27 Rajaofetra N, Passagia JG, Marlier K *et al.* Serotoninergic, noradrenergic, and petidergic innervation of Onuf's nucleus of normal and transected spinal cords of baboons (*Papio papio*). *J. Comp. Neurol.* 1992; **318**: 1–17.
28 Thor KB. Serotonin and norepinephrine involvement in efferent pathways to the urethral rhabdosphincter: implications for treating stress urinary incontinence. *Urology* 2003; **62**: 3–9.
29 Kaiho Y, Kamo I, Chancellor MB *et al.* Role of noradrenergic pathways in sneeze-induced urethral continence reflex in rats. *Am. J. Physiol. Renal. Physiol.* 2007; **292**: F639–46.
30 Kamo I, Torimoto K, Chancellor MB *et al.* Urethral closure mechanisms under sneeze-induced stress condition in rats: a new animal model for evaluation of stress urinary incontinence. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2003; **285**: R356–65.

Editorial Comment

Editorial Comment from Dr Saito and Dr Shimizu to Propiverine increases urethral wall catecholamine levels and bladder leak point pressure in rats

Many reports have shown that muscarinic receptor antagonists have been used and are thought to be effective against overactive bladder, but not for stress urinary incontinence (SUI).[1] In Japan, only clenbuterol hydrochloride, a selective β2-adrenergic agonist, is available to prescribe for SUI patinents.[2,3] In addition, tricyclic antidepressants, such as imipramine hydrochloride, are also used for off-label use.[4] Interestingly, previous manuscripts have reported the efficacy of propiverine, an anticholinergic drug with calcium antagonistic action, against pure SUI patients in Japan.[5] In the present study, Nishijima *et al.* tried to investigate the mechanism through which propiverine is effective for treating SUI.[6] The authors found that intravenous injection of propiverine increased the leak point pressure in rats with vaginal distention. The mechanism of this effect could be that propiverine acted like a noradrenaline re-uptake inhibitor, and subsequently increased noradrenaline and/or dopamine levels in the plasma, cerebrospinal fluid, and urethral wall perfusion fluid. The authors concluded that the inhibition of noradrenaline re-uptake by propiverine mainly occurs at the urethral level, and partially at the central nervous system; furthermore, it might stimulate the smooth muscles of the bladder neck and proximal urethra through α1-adrenergic receptors, as well as it might stimulate the striated muscles of the urethra and pelvic floor by an activation of the spinal motoneurons. These data clearly suggest a new mechanism for the protective effect of propiverine in the lower urinary tract in the rat. A limitation of this study was whether their new finding; that is, propiverine's action as a noradrenaline re-uptake inhibitor, can be applied to humans in clinically used-doses, considering that it is quite difficult to confirm the new finding in humans.

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Conflict of interest

None declared.

References

1 Yamaguchi O. Latest treatment for lower urinary tract dysfunction: therapeutic agents and mechanism of action. *Int. J. Urol.* 2013; **20**: 28–39.
2 Tsakiris P, de la Rosette JJ, Michel MC, Oelke M. Pharmacologic treatment of male stress urinary incontinence: systematic review of the literature and levels of evidence. *Eur. Urol.* 2008; **53**: 53–9.
3 Ushiroyama T, Ikeda A, Ueki M. Clinical efficacy of clenbuterol and propiverine in menopausal women with urinary incontinence: improvement in quality of life. *J. Med.* 2000; **31**: 311–9.
4 Lin HH, Sheu BC, Lo MC *et al.* Comparison of treatment outcomes for imipramine for female genuine stress incontinence. *Br. J. Obstet. Gynaecol.* 1999; **106**: 1089–92.
5 Sugaya K, Sekiguchi Y, Satoh T *et al.* Effect of propiverine hydrochloride on stress urinary incontinence. *Int. J. Urol.* 2014; **21**: 1022–5.
6 Nishijima S, Sugaya K, Kadekawa K, Ashitomi K, Ueda T, Yamamoto H. Propiverine increases urethral wall catecholamine levels and bladder leak point pressure in rats. *Int. J. Urol.* 2016; **23**: 93–9.

Editorial Comment

Editorial Comment from Dr Negoro to Propiverine increases urethral wall catecholamine levels and bladder leak point pressure in rats

The article by Nishijima *et al.* showed that propiverine, known as an antimuscarinic and Ca-antagonistic agent, increased leak point pressure in rats by acting as a noradrenaline re-uptake inhibitor like imipramine and nisoxetine, which mainly occurred in the urethra through α1-adrenoceptor.[1] Elucidating the pharmacological action of propiverine on stress urinary incontinence is appreciated, as treating stress urinary incontinence pharmacologically is not straightforward, especially in patients suffering after radical prostatectomy, and progress in this field has been awaited.

This basic research is important to support the clinical effect of propiverine on stress urinary incontinence in a human study, though there are some points to be carefully considered, because differences in the pharmacological effect