Mechanisms Underlying Mechanical Responses to *Ephedra herb* of Isolated Rabbit Urinary Bladder and Urethra, a Possible Stress Urinary Incontinence Therapeutic

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Abstract. To compare the mechanisms underlying mechanical responses to ephedrine and *Ephedra herb*, a main component of *Kakkon-to*, in isolated male and female rabbit urinary bladder and urethral strips, responses of isolated strips to the agents were recorded in organ bath systems. Ephedrine and *Ephedra herb* relaxed the female urinary bladder to the similar extent. These relaxations are reversed to contractions by timolol. In the presence of timolol, ephedrine produced less contraction of urethral strips in the female than those in the male; this contraction was abolished by prazosin. *Ephedra herb* contracted the female urethra less than that of the male, and the contraction was stronger than that by ephedrine. The contraction caused by *Ephedra herb* in strips treated with timolol was significantly inhibited by prazosin. The prazosin-resistant contraction of the female urethra was greater than that of the male. Quinacrine, a phospholipase A₂ inhibitor, indomethacin, and AA861, a 5-lipoxygenase inhibitor, inhibited the contraction. The contraction was inhibited by ZK 158252, a leukotriene (LT) B₄-receptor antagonist. These findings suggest that *Ephedra herb* contracts the urethra via arachidonic acid metabolites together with α₁-adrenoceptor stimulation. The metabolites produced by 5-lipoxygenase may stimulate LTB₄, but not CysLt₂, receptors. These contractile components induced by *Ephedra herb* and *Kakkon-to* might be effective for the treatment of stress urinary incontinence.

Keywords: stress urinary incontinence, *Ephedra herb*, ephedrine, urethral contraction, arachidonic acid metabolite

Introduction

Women have higher rates of urinary incontinence than men, which increase with age; one third of women older than 65 years of age have some degree of incontinence (1). Urinary incontinence is roughly classified into urge and stress incontinence. The combination of non-medical therapy including biofeedback-assisted behavioral training and medication of anticholinergic agents, oxybutynin and tolterodine, for urge incontinence results in a better control of the incontinence than either treatment alone (2). On the other hand, when treating female patients with stress urinary incontinence (SUI), physicians should consider various therapies including pelvic floor muscle exercises, intravaginal support devices, pessaries, urethral occlusion inserts, medication, and surgery (3). As one of the medications, estrogens have been used for the treatment of urinary incontinence since as early as 1941 (4). However, in a recent analysis of the medication, little benefit of estrogens for SUI was found (5). Furthermore, α-adrenoceptor agonists have been found to be effective for SUI in clinical trials, but the agonists lack exclusive selectivity for urethral α-adrenoceptors and may cause elevation of blood pressure, palpitation, sleep disturbance, and headache (6). Thus, the Food and Drug Administration approves no drugs for SUI at the present time (3).

*Kakkon-to*, a traditional Chinese herbal medicine, has been used for the treatment of common cold, tonsillitis, and chronic inflammatory diseases (7).
Recently, Japanese gynecologists noticed on the basis of their experiences that Kakkon-to is effective for SUI because it decreases the scores of the international consultation on incontinence questionnaire short form composed of 3 categories: frequency, severity, and quality-of-life impact of urinary incontinence (8). However, mechanisms of actions on SUI of Kakkon-to are unknown.

Ephedra herb is a major component of Kakkon-to. Ephedrine extracted from Ephedra herb is classified as a miscellaneous adrenergic agonist (9). Ephedrine was found to be effective for SUI in clinical trials (10).

Therefore, in the present study, we compared the mechanisms underlying mechanical responses of isolated urinary bladder and urethra to ephedrine and Ephedra herb in male and female Japanese White rabbits in order to explain a possible mechanism by which Kakkon-to clinically works as a therapeutic for female SUI.

Materials and Methods

Animals

Eight male and twenty female Japanese White rabbits, weighing 3.0 – 4.0 kg, were used for the present study. The Animal Care and Use Committee at Shiga University of Medical Science approved the use of rabbit urinary bladder and urethra in this study.

Mechanical response

Under deep general anesthesia with ketamine (20 mg/kg, i.m.) and sodium pentobarbital (25 mg/kg, i.v.), Japanese White rabbits were killed by bleeding from common carotid arteries. The urinary bladder was isolated, and the largest part of the corpus of the bladder was transversely cut into several open-ring strips of approximately 20-mm length. The urethra close to the ostum urethrae was isolated and cut into an open-ring strip of approximately 20-mm length. The strips were fixed vertically between hooks in an organ chamber containing a modified Ringer-Locke solution, as previously reported (11). The resting tension was adjusted to 1.5 g.

Isometric contractions and relaxations were displayed on an ink-writing recorder. The contractile response to 5 mM Ba\(^{2+}\) was obtained first, and the urinary bladder and urethral strips were repeatedly washed with fresh media and equilibrated. The strips of urinary bladder were partially contracted with prostaglandin (PG) F\(_{2\alpha}\) (0.2 – 1 × 10\(^{-6}\) M), the contraction being in the range between 35% – 40% of the contraction induced by 5 mM Ba\(^{2+}\). Concentration–response curves for ephedrine and Ephedra herb were obtained by adding the drug directly to the bathing media in cumulative concentrations. The initial responses to the drugs were compared in the absence or presence of timolol. At the end of each series of the experiments, papaverine (10\(^{-4}\) M) was applied to attain the maximum relaxation. Relaxations induced by ephedrine and Ephedra herb in the urinary bladder strips are expressed relative to those induced by papaverine as 100% and contractions induced by the drugs after treatment with \(\beta\)-blocker in the urinary bladder are expressed relative to those induced by 5 mM Ba\(^{2+}\). In the case to compare the urethral responses to ephedrine and Ephedra herb between males and females, the contractions induced by the drugs are expressed relative to those induced by 5 mM Ba\(^{2+}\). After reproducibility of the contractions caused by ephedrine and Ephedra herb under treatment with prazosin and timolol was determined in the case of urethra, the strips were treated with blocking agents. In order to evaluate these blocking agents contractions induced by Ephedra herb are expressed relative to the maximal contraction induced by 300 \(\mu\)g/ml of Ephedra herb in the absence of blocking agents.

Drugs and statistics

The results shown in the text and figures are expressed as mean values ± S.E.M. Statistical analyses were made using Student’s unpaired t-test for two groups. Drugs used were PGF\(_{2\alpha}\) (Pfizer Inc., Tokyo); ephedrine hydrochloride (Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya); Ephedra herb (an extract of Ephedra sinica, Lot No. 2991037010; Tsumura & Co., Tokyo); prazosin hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka); atropine sulfate (Tanabe Seiyaku Co., Ltd., Osaka); yohimbine hydrochloride (Nacalai Tesque Inc., Kyoto), methysergide hydrogen maleate (Novartis AG, Basel, Switzerland); \(d\)-chlorphenylamine maleate (Schering-Plough Co., NJ, USA); AA861 (Takeda Pharmaceutical Co., Ltd., Osaka); montelukast (Merck & Co., Inc., NJ, USA); ZK 15252 (Bayer HealthCare, Berlin, Germany); timolol maleate, quinacrine dihydrochloride, and indomethacin (Sigma Chemical, St. Louis, MO, USA); and papaverine hydrochloride (Dainippon, Osaka). The powder of Ephedra herb was sufficiently mixed with distilled water, and the solution was filtrated with filter paper (Tokyo Roshi Kaisha, Ltd., Tokyo). Thereafter, the filtrate was diluted with distilled water in order to adjust the concentration to 10 mg/ml. ZK 15252 was dissolved in the solvent (4 ml of 99.5% ethanol + 0.05 ml of 1 M NaOH + 36 ml of distilled water).
Effect of Ephedra herb on Urinary Tract

Results

Response of urinary bladder strips from the female rabbits to ephedrine and Ephedra herb

Ephedrine and Ephedra herb relaxed the urinary bladders of female rabbits in a concentration-dependent manner. The maximal relaxations caused by these drugs were almost the same, and they were reversed to a slight contraction by treatment with $10^{-6}$ M timolol, a $\beta$-blocker (Fig. 1).

Response of urethral strips from male and female rabbits to ephedrine and Ephedra herb

Ephedrine contracted urethral strips of male and female rabbits in a concentration-dependent manner, and the magnitude of the contractions was not significantly different between male and female rabbits (Fig. 2, top left). Under treatment with $10^{-6}$ M timolol, contractile responses to ephedrine of the female urethra were significantly less than those of the male (Fig. 2, top center). The ephedrine-induced contractions in the male and female rabbits were abolished by additional treatment with $10^{-5}$ M prazosin, an $\alpha_1$-adrenoceptor blocker (Fig. 2, top right).

Ephedra herb contracted the urethral strips both of male and female rabbits in a concentration-dependent manner, and the magnitude of contractions in the female rabbits was significantly less than those in the male with and without $10^{-6}$ M timolol (Fig. 2, bottom left and center). Under treatment with $10^{-6}$ M timolol, the contractile responses to Ephedra herb in the male and female rabbits were not abolished by $10^{-5}$ M prazosin, and the magnitude of the contractions at $30 \mu g/ml$ of Ephedra herb in the female was slightly but significantly greater than that in the male ($10.8 \pm 4.1\%$ (n = 5) in the female and $1.2 \pm 0.5\%$ (n = 5) in the male; Fig. 2, bottom right).

Discussion

The isolated urinary bladder strips from female rabbits were relaxed by ephedrine and Ephedra herb almost to the same extent. The relaxations were reversed to slight contractions by timolol. These findings indicate that both drugs relax the rabbit urinary bladder by stimulation of $\beta$-adrenoceptors as seen in human urinary bladder (20, 21), suggesting that $\beta$-adrenergic agonists promote the urine storage by increasing urinary bladder capacity.

Substantial pharmacological and physiological evidences indicate that urethral tone is mainly regulated by $\alpha$-adrenoceptors (22). In the presence of timolol,
Fig. 2. Contractile responses to ephephrine (top panel) and *Ephedra herb* (bottom) in male (circle) and female (triangle) rabbit urethral strips without treatment (left), with $10^{-6}$ M timolol (center) and $10^{-6}$ M timolol plus $10^{-5}$ M prazosin (right). Contractions induced by 5 mM Ba$^{2+}$ were taken as 100%. Numbers in parentheses indicate the number of experiments. Comparisons were made using the unpaired *t*-test. Bars = S.E.M. *$P<0.05$, †$P<0.01$, ‡$P<0.005$, vs male.

Fig. 3. Real tracings of the contractile responses to *Ephedra herb* in female rabbit urethral strips exposed to control media (Control, A, B, and C, left) and those containing $3 \times 10^{-5}$ M quinacrine (A, right), $10^{-6}$ M indomethacin (B, right), or $10^{-5}$ M AA861 (C, right) in the presence of $10^{-6}$ M timolol plus $10^{-5}$ M prazosin. Contractile responses to *Ephedra herb* in female rabbit urethral strips without (circle) and with (triangle) $3 \times 10^{-5}$ M quinacrine (D), $10^{-6}$ M indomethacin (E), or $10^{-5}$ M AA861 (F) in the presence of $10^{-6}$ M timolol plus $10^{-5}$ M prazosin. Contractions induced by 300 $\mu$g/ml of *Ephedra herb* in the control media were taken as 100%. *n* = number of strips. Comparisons were made using the unpaired *t*-test. Bars = S.E.M. *$P<0.05$, †$P<0.01$, ‡$P<0.005$, ¶$P<0.00001$, vs Control.
Ephedra herb also contracted the urethral strips, and the contraction was more potent than that by ephedrine both in the male and female rabbits regardless of the \( \alpha \)-adrenoceptor blockade (Fig. 2). Treatment with prazosin abolished the ephedrine-induced contraction, but not the Ephedra herb—induced contraction. Thus, Ephedra herb is found to produce contraction via a mechanism other than stimulation of \( \alpha \)-adrenoceptors. The contraction of the urethral strips was not affected by a sufficient concentration of atropine, yohimbine, methysergide, or chlorphenylamine under the blockade of \( \alpha \)- and \( \beta \)-adrenoceptors, suggesting that the contraction is not mediated via stimulation of muscarinic, \( \alpha_2 \), 5-HT\( \_1 \) + 5-HT\( \_2 \), or \( H\_1 \) receptors. Quinacrine, indomethacin, and AA861 significantly inhibited the contraction, suggesting that cyclooxygenase metabolites and 5-lipoxygenase metabolites are involved in the response. In fact, prostaglandins have been reported to be a constricting agent in the rabbit urethra (24), but the involvement of 5-lipoxygenase metabolites have not been reported. In the present study, the contraction resistant to prazosin was inhibited by ZK 158252, a leukotriene (LT) \( \beta\_4 \) receptor antagonist (18), but was not by montelukast, a CysLT\( \_1 \)-receptor antagonist, suggesting that LT\( \beta\_4 \) but not LTC\( \_4 \) or LTD\( \_4 \) is involved in the response. Bouchelouche et al. (19) has reported that LTD\( \_4 \) produced a contraction followed by an increase in [Ca\( ^{2\,+} \)], which was inhibited by montelukast, in human detrusor smooth muscles. However, receptor functions of leukotrienes in the urethra are not precisely known at this moment. It has been reported that LT\( \beta\_4 \), known as a potent leukocyte chemoattractant, produced contractions in the guinea-pig trachea, bronchus, and pulmonary artery and in the human pulmonary artery (25). Taken together, Ephedra herb may contract the urethra by producing several metabolites of arachidonic acid besides by stimulating \( \alpha \)-adrenoceptors. The metabolites produced by 5-lipoxygenase probably stimulate receptors of LT\( \beta\_4 \) but not of LTD\( \_4 \).

As described earlier, Japanese gynecologists obtained a nice clinical result with little adverse effects when Kakkon-to was administrated to the SUI female patients. The dosage of the herbal medicine used was 5.0 – 7.5 g daily. Kakkon-to contains 16.7% of Ephedra herb, and Ephedra herb contains less than 1.0% of ephedrine and some other \( \alpha \)-adrenoceptor stimulants such as phenylpropanolamine, pseudophedrine, and \( N \)-methyl-ephedrine. If this is the case, the effective dose of Kakkon-to for treatment of SUI contains 13.0 mg
ephrine at most, and the adverse effect of ephrine, which was reported to be induced at doses higher than 40 mg daily (10), may not be induced. In the present study, *Ephedra herb* is found to be effective for SUI by mechanisms other than those that ephrine and other α₁-adrenoceptor stimulants elicit. In this context, *Ephedra herb* itself might be a possible therapeutic as long as proper doses are used.

Since *Kakkon-to* contains other substances than *Ephedra herb*, it is difficult to fully explain the clinical merit of *Kakkon-to* for urinary incontinence. However, *Ephedra herb* itself is difficult to use for the treatment because of the adverse reaction. Therefore, the mechanism associated with arachidonic acid metabolites may be important to create a new strategy for the treatment of SUI.

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References


