Tzu Chi Medical Journal 2023; 35 (3): 253-259

Original Article

The differences in the adrenergic receptors of proximal urethra between sexes

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Submission : 16-Aug-2022
Revision : 08-Sep-2022
Acceptance : 03-Nov-2022
Web Publication : 22-Feb-2023

Abstract

Objectives: The bladder and urethra work as a physiologically functional unit to facilitate continence in the storage and voiding phase. Sex differences have been found in the urethral contraction in response to α-adrenergic receptor activation. This study aimed to investigate the role of adrenergic receptors in the proximal urethra of male and female mice. **Materials and Methods:** Urinary bladder and proximal urethral smooth muscle (USM) samples from male and female C57BL/6 mice were isolated and mounted in an organ bath. **Results:** Acetylcholine-induced contraction of the urinary bladder was compared in male and female mice. Phenylephrine and norepinephrine (NE) induced little contraction at a lower concentration, but a relaxing phase of female proximal USM was observed at a higher concentration. This contraction profile was inhibited by N^G-nitro-L-arginine, lidocaine, and capsaicin. In addition, the NE-induced contraction was greater in the incubation of propranolol than that of L-NNA or lidocaine. These results suggested that the β-adrenoceptor may be the dominant receptor of female proximal USM, and the activity of calcitonin gene-related peptide sensory nerves and nitrergic nerves may pose an anti-contraction effect on the proximal urethra in female mice. **Conclusion:** β-adrenoceptor may be the dominant receptor of female proximal USM. The use of β-adrenergic receptor blocker agents might have the potential for the treatment of female voiding dysfunction.

Keywords: *Adrenoceptor, Proximal urethra, Sex differences*

Introduction

The bladder and urethra work as a functional unit physiologically, and the autonomic nervous system plays a key role in the regulation of the lower urinary tract (LUT) function. During the phase of urine storage, adrenergic nerves play a crucial role [1,2]. Norepinephrine (NE) released from adrenergic nerve endings relaxes the bladder body by increasing intracellular cyclic adenosine monophosphate through β-adrenergic receptors that are widely expressed in the bladder body, and NE contracts the bladder base and proximal urethra through α_1-adrenoceptors to prevent premature bladder emptying [3]. The urethra consists of smooth and striated muscle layers. Relaxation of both muscles is essential for effective voiding; similarly, contraction of both muscles is necessary for maintaining urinary continence [4,5]. The adrenergic nerves form a plexus that usually runs parallel to the long axes of the smooth muscle cells [6,7], and expressions of α_1-adrenoceptors in the proximal urethral smooth muscle (USM) play a major role in urinary continence [7].

There are three subtypes of α_1-adrenoceptors present in peripheral tissues: α_{1A}, α_{1B}, and α_{1D} [8], which are expressed in the urinary bladder, and mediate contraction of the detrusor in response to phenylephrine (PE) and NE. The α_{1A}-adrenoceptors also mediate prejunctional facilitation [9] on cholinergic nerve terminals, which facilitate the release of both acetylcholine and noncholinergic nonadrenergic transmitter [10], causing detrusor contraction. In addition, the α_1-adrenoceptors are functionally expressed by capsaicin-sensitive, primary sensory neurons of the urinary tract [11].

A greater contraction of urethra to α_2-adrenoceptors agonist was found in female rabbits than in male rabbits due to a higher density of α_2-adrenoceptors in female urethra [12,13].

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Access this article online

Quick Response Code:

Website: www.tcmjmed.com

DOI: 10.4103/tcmj.tcmj_221_22

How to cite this article: Hsieh PC, Chang SJ, Chang HH, Yang SS. The differences in the adrenergic receptors of proximal urethra between sexes. Tzu Chi Med J 2023;35(3):253-9.

A marked expression of α_{1A}-adrenoceptors subtypes was found in the USM of male mice than in female USM [14]. Furthermore, tyrosine hydroxylase (sympathetic nerve marker) also exhibited a higher mRNA expression in the urethra of male mice than in female urethra [14].

Urinary incontinence has a substantially negative impact on health-related quality of life and is associated with considerable personal and societal expenditure [15]. The prevalence of urine incontinence is significantly higher in women than in men. In addition to anatomical differences, whether the adrenergic receptors play a role is unclear. Therefore, the aim of this study was to investigate the role of adrenergic nerve receptors in modulating USM contraction and to explore the differences between male and female mice.

Materials and Methods

Experimental animals

Experiments were conducted with approval of the Institutional Animal Care and Use Committee this study IACUC number is 109-006 at Taipei Tzu Chi Hospital. Female/male 5-week C57BL/6 mice were maintained under a controlled light (12-h light/dark cycles from 7:00 AM to 7:00 PM) and temperature (21°C–23°C). The 5-week-old C57BL/6 mice were fed a normal diet containing 5.9% fat, and their body weight was measured every week. When they were sacrificed, they were anesthetized, and samples of blood, feces, and internal organs were collected.

Tissue preparation

The C57BL/6 mice of 10–13 weeks were sacrificed by cervical dislocation after anesthesia with urethane (500 mg/kg, ip) and chloralose (50 mg/kg, ip). The urinary bladder and urethra were dissected and placed in oxygenated (95% O_2 and 5% CO_2) Krebs' bicarbonate solution (KBS; measured in mM) at 4°C. It was composed of NaCl 117, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.1, and calcium disodium ethylenediaminetetraacetate 0.023. The bladder and urethra were dissected free as a block and immersed in a petri-dish containing the KBS [Figure 1]. Next, the surrounding tissue was carefully removed, and a 3-mm rings proximal urethra was excised from the bladder neck and external urethral sphincter [16,17]. The striated muscle of the external urethral sphincter was carefully removed during the dissection. Bladder strips and urethral rings were mounted in 20 mL organ baths containing KBS continuously bubbling with a mixture of 95% O_2/5% CO_2 at pH 7.4 and at 37°C. Isometric tension change was measured by an isometric transducer (FT03C; Grass) and a Powerlab polygraph (ADInstruments Pty Ltd., Castle Hill, Australia). The equilibration period was 60 min, and the bathing medium was changed every 15 min until the start of the experiments.

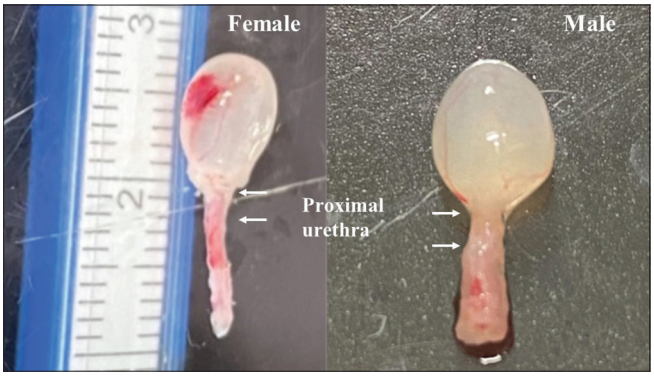

Figure 1: Photo shows the dissected male and female proximal urethra (between the two arrows)

Tension recording for urinary bladder

The urinary bladder strips were isolated and cleaned off surrounding tissues under a dissecting microscope. They were then mounted on a stainless-steel rod and a platinum wire in a tissue bath containing 20 mL KBS. This KBS was gassed with 95% oxygen and 5% carbon dioxide at 37°C. The urinary bladder strips were equilibrated in the KBS for 60 min and mechanically stretched to a resting tone of 0.5 gm. After equilibration, the active muscle tone of urinary bladder strips was induced by acetylcholine (10^{-9}–10^{-5} M).

Tissue bath wire myography for urethral rings

Urethral rings were isolated and cleaned off surrounding tissues under a dissecting microscope. They were then mounted on a stainless-steel rod and a platinum wire in a tissue bath containing 20 mL KBS. This KBS was gassed with 95% oxygen and 5% carbon dioxide at 37°C. The urethral rings were equilibrated in the KBS for 60 min and mechanically stretched to a resting muscle tone of 0.2 g.

In Step 1 after equilibration, the active tension of urethral rings was induced by cumulative applications of PE (10^{-9}–10^{-5} M). In Step 2, the urethral rings were precontracted with PE (10 μM), and then relaxation effects were induced by acetylcholine (10 μM) and nicotine (50 μM). Between Steps 1 and 2, there were 45 min of washes with the KBS. In Step 3, after the completion of washes, silodosin (0.1 μM), lidocaine (0.1 mM), N^G-nitro-L-arginine (100 μM), or guanethidine (10 μM) was added 15 min before the application of PE (10 μM) to induce contraction. Then, the effects of relaxation were recorded. In Step 4, the maximal contraction of the urethral rings was induced by KCl (100 mM). Only one isolated urethral ring per animal was used in the tissue bath study.

Chemical denervation with capsaicin

To deplete calcitonin gene-related peptide (CGRP) in CGRP-containing sensory nerves in the USM strips, urethral segments were incubated in KBS (37°C) containing capsaicin (a CGRP depleter, 1 μM) for 20 min, and then rinsed for 60 min in capsaicin-free Krebs solution.

Drugs used and statistical nalysis

The following chemicals were used: NaCl, $NaHCO_3$, KCl, $CaCl_2$, $MgCl_2$, glucose, NaH_2PO_4, ethylenediaminetetraacetate, nicotine, acetylcholine, lidocaine, silodosin, NE, capsaicin, and PE (all from Sigma-Aldrich, ST Louis, MO, USA). A paired samples *t*-test was used to compare the difference in the same strip. An analysis of variance followed by *post hoc* tests using Bonferroni was used to compare the difference between different strips. All values are presented as mean ± standard error of the mean. $P < 0.05$ was considered statistically significant.

Results

Urinary bladder and urethral contractions in different sex

Concentration-response curves to accumulative applications of acetylcholine (10^{-9}–10^{-5} M) were produced to compare contraction profiles of the urothelium-intact bladder detrusor in male and female mice. There were no significant differences in the contraction profiles between male and female mice [Figure 2a and b, $n = 6$, $P > 0.05$]. In contrast, exogenous applications of PE (10^{-9}–10^{-5} M) induced contractions in the proximal USM such that it induced little contraction at lower concentrations followed by a relaxing phase at higher concentrations in female mice [Figure 2c and d, $n = 10$]. The results showed that exogenous applications of PE (10^{-9}–10^{-5} M) induced greater contraction in the proximal urethral rings of male mice than in those of female mice [Figure 2d, $n = 10$, $P > 0.05$].

Male proximal urethral contractions to phenylephrine and norepinephrine

Contractile responses of the USM were elicited by accumulative applications of PE (10^{-9}–10^{-5} M) in a concentration-dependent manner [Figure 3a, $n = 6$]. Exogenous applications of NE (10^{-9}–10^{-5} M) induced contraction in the proximal USM in a fashion similar to PE [Figure 3a, $n = 6$, $P > 0.05$]. However, the NE-induced contraction was significantly inhibited by the pretreatment with silodosin [0.1 μM, Figure 3b, $n = 5$, $P < 0.05$].

Effect of propranolol (β-adrenoceptor blocker) in norepinephrine-induced contraction on proximal urethral smooth muscle rings

The cumulative addition of NE (10^{-9}–10^{-5} M) produced little contraction, and the contraction shifted to a relaxing phase in isolated rings at higher concentrations [Figure 4a and b, $n = 5$]. Prior incubation of the urethral tissue with propranolol (10 μM) significantly increased the NE-induced contraction [Figure 4b, $n = 5$, $P < 0.05$]. In addition, the NE-induced contraction was significantly greater when incubated with propranolol than with N^G-nitro-L-arginine or lidocaine [Figure 4b, $n = 5$, $P < 0.05$].

Effect of nitric oxide synthase inhibitor and lidocaine in nicotine- and acetylcholine-induced relaxation in male urethral smooth muscle rings

In the presence of active muscle tone induced by PE (10 μM), nicotine (50 μM)-and acetylcholine (10 μM)-induced relaxation in the USM rings was significantly inhibited by N^G-nitro-L-arginine [100 μM, Figure 5a, $n = 5$, $P < 0.05$] and lidocaine [1 μM, Figure 5b, $n = 5$, $P < 0.05$]. In female mice, the ACh-and nicotine-induced relaxations were inhibited by N^G-nitro-L-arginine [Figure 5c].

Effect of calcitonin gene-related peptide depleter in nicotine- and acetylcholine-induced relaxation in the urethral smooth muscle rings

The USM rings were pre-incubated with capsaicin (1 μM) for 20 min and then rinsed for 60 min in the capsaicin-free

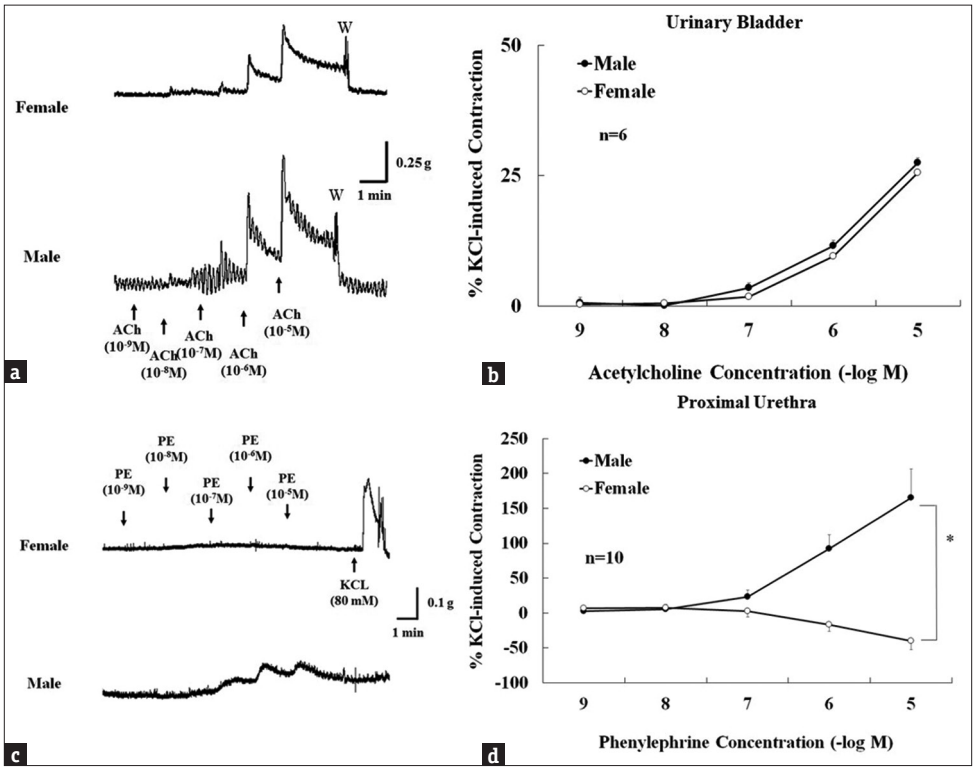

Figure 2: Urinary bladder and proximal USM contraction in male and female mice. A representative tracing in (a) shows that in the absence of active muscle tone, the bladder strips contract upon application of acetylcholine (10^{-9}–10^{-5} M) in male and female mice. These results are summarized in (b). A representative tracing in (c) shows that in the absence of active muscle tone, the USM rings contract upon application of (PE, 10^{-9}–10^{-5} M) in male and female mice. These results are summarized in (d). The magnitude of contraction was calculated as the percent of KCl-induced maximum contraction. n, number of experiments. Values are means ± SEM. Asterisk indicates a significant difference in Bonferroni posttests following ANOVA (*$P < 0.05$). USM: Urethral smooth muscle, PE: Phenylephrine, SEM: Standard error of the mean, ANOVA: Analysis of variance

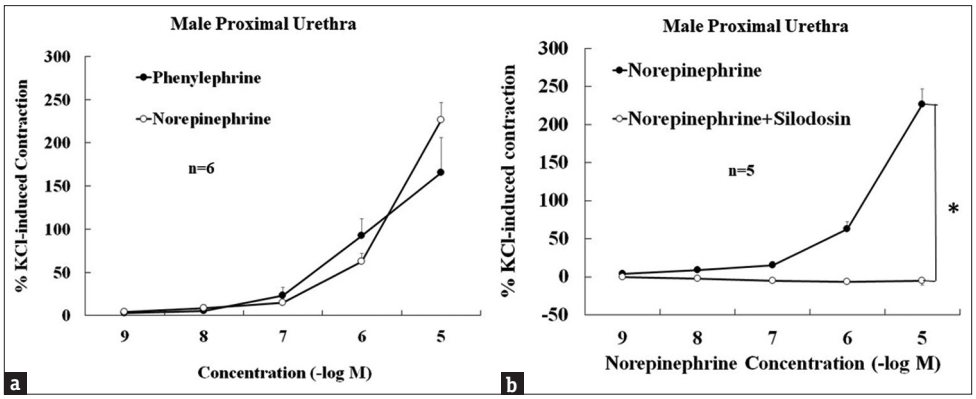

Figure 3: Effect of α_1-adrenoceptor antagonist silodosin on norepinephrine-induced proximal USM rings contraction in male mice. PE-and NE-induced concentration dependent constrictions of proximal USM rings were performed in proximal USM in the absence of active muscle tone (a). Constrictions induced by NE were abolished by silodosin (0.1 μM) (b). The magnitude of contraction was calculated as the percent of KCl-induced maximum contraction. n, number of experiments. Values are means ± SEM. Asterisk indicates a significant difference in Bonferroni posttests following ANOVA (*$P < 0.05$). USM: Urethral smooth muscle. PE: Phenylephrine, NE: Norepinephrine, SEM: Standard error of the mean, ANOVA: Analysis of variance

KBS. In the presence of active muscle tone induced by PE (10 μM), nicotine (50 μM)-, and acetylcholine (10 μM)-induced relaxation in the USM rings was significantly inhibited by pretreatment with capsaicin [Figure 5d, $n = 5$, $P < 0.05$].

Effect of guanethidine in nicotine- and acetylcholine-induced relaxation in the urethral smooth muscle rings

The USM rings were preincubated with guanethidine (10 μM) for 15 min. In the presence of active muscle tone induced by PE (10 μM), nicotine (50 μM)-, and acetylcholine (10 M)-induced relaxation in the USM rings was significantly inhibited by guanethidine [10 μM, Figure 5d, $n = 5$, $P < 0.05$].

Discussion

This study demonstrated that urinary bladder contractility was comparable between the sexes in mice, while the contraction profiles of the proximal urethra were different between sexes in mice. Both contractors, PE and NE, induced a similar contraction profile in the female proximal urethra. Lower concentrations (10^{-9}–10^{-5} M) caused little contraction, but higher concentrations (10^{-7}–10^{-5} M) led to a relaxing phase. In addition, NE-induced contraction of the female proximal urethra was inhibited by N^G-nitro-L-arginine and capsaicin, suggesting that nitrergic and sensory CGRP nerve activity could affect the USM basal muscle tone. Therefore, the β-adrenoceptor may be a dominant receptor on the proximal USM that reverses NE-induced contraction to relaxation as propranolol (β-adrenoceptor blockers) significantly increased the NE-induced contraction.

The urethra plays a major role in the LUT function [18], and USM tension is one of the key factors in maintaining intraurethral pressure and contributing to continence [19]. The prevalence of urinary incontinence is significantly higher in women than in men [20,21]. In case of stress urinary incontinence, the strength of both the urethral striated and smooth muscles is significantly impaired [22-25]. These urethral muscles innervated by adrenergic nerves through α_1-adrenoceptors help maintain resting urethral pressure [14,26,27].

PE-induced contraction of the proximal USM in male mice was greater than in female mice [Figure 3]. In addition, NE-induced, concentration-dependent contraction was inhibited by silodosin [α_{1A}-blocker, Figure 3b]. These results suggested that α_{1A}-adrenoceptor-mediated NE-induced contraction. Previous studies indicated that the proximal USM of male mice yielded greater α_{1A}-adrenoceptor-induced contractions and had more α_{1A}-adrenoceptor distribution density and tyrosine hydroxylase (sympathetic nerve marker) mRNA expression than that of female mice. The lower adrenergic receptors density in female proximal urethra may contribute to the higher incidence of urinary incontinence in women [14]. Female rats with stress urinary incontinence had a significantly decreased neuronal nitric oxide (nNOS)/TH-positive nerves ratio in the smooth muscle layers of the urethra [28], suggesting that incontinence would enhance the expression of tyrosine hydroxylase on the USM [26].

PE and NE induced a biphasic contraction in female proximal USM. It was followed by a relaxing phase, which was inhibited by N^G-nitro-L-arginine, lidocaine, and capsaicin [Figure 4]. These results suggested that the activity of CGRP sensory nerves and nitrergic nerves may pose an anti-contraction effect on the proximal urethra in female mice. In addition, the NE-induced relaxing phase was inhibited more by propranolol than by lidocaine, suggesting that the β-adrenoceptors were present on the proximal USM and may play a dominant role [Figure 4].

In addition to urethra, the effect of β-adrenergic agonist in voiding, the inter-micturition interval was enhanced and decreased nonvoiding contractions by bambuterol (β_2-agonist, 10 mg/kg) treatment. Previous studies indicated that the CL316243 (β_3-agonist) significantly decreases the amplitude of both micturition and nonvoiding contractions [29], but β_2-adrenoceptors in female mouse bladder inhibit electrical field stimulation-induced contractions [30]. Therefore, β-adrenergic agonists can enhance intermicturition interval and decrease nonvoiding contractions, in female mice. Another study indicated that the procaterol (β_2-agonist) clearly decreased urethral pressure but CL316243 (β_3-agonist)

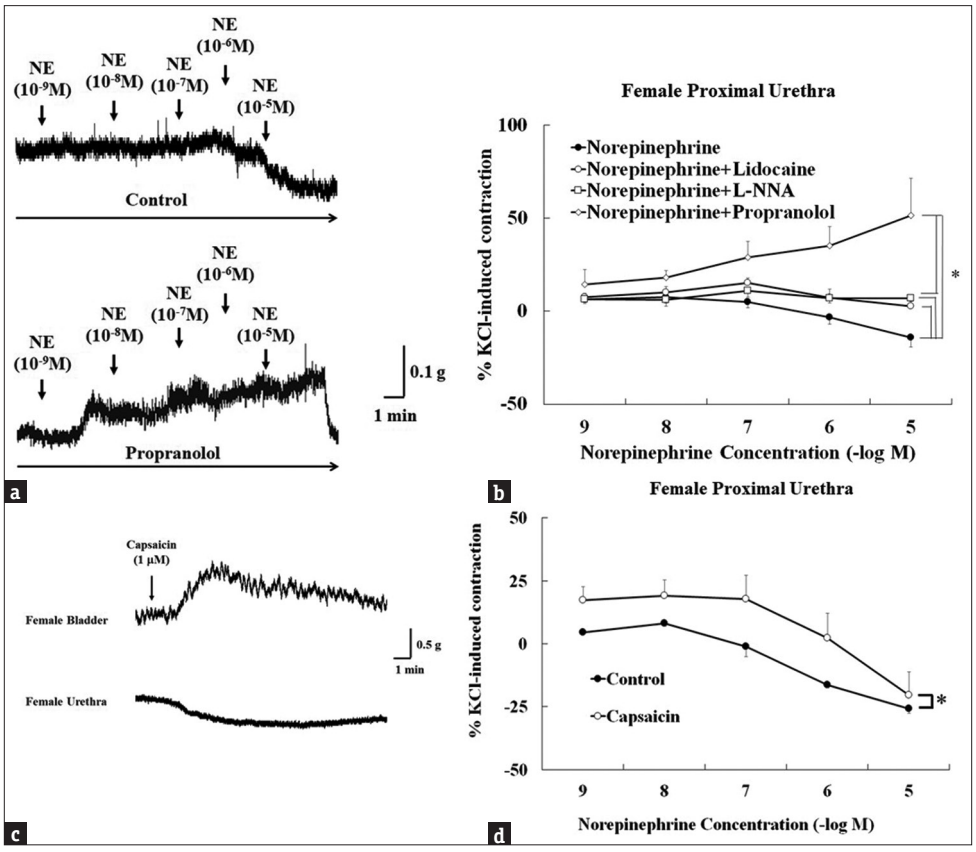

Figure 4: Effect of nitric oxide synthase inhibitor, and lidocaine, and CGRP deplete, and propranolol on proximal USM rings relaxation in female mice. A representative tracing in (a) shows that NE-induced concentration dependent constrictions of USM rings were enhanced by propranolol (10 μM). NE -induced concentration dependent constrictions of USM rings were enhanced by lidocaine (1 μM), and N^G-nitro-L-arginine (100 μM), and propranolol (10 μM) (b). A representative tracing in (c) showing that in the absence of active muscle tone, the UB strips contract and USM rings relax on application of capsaicin (1 μM). Norepinephrine-induced concentration-dependent constrictions of USM rings were enhanced by capsaicin (1 μM) (d). n, number of experiments. Values are means ± SEM. Asterisk indicates a significant difference in Bonferroni posttests following ANOVA (*$P < 0.05$). USM: Urethral smooth muscle. NE: Norepinephrine, SEM: Standard error of the mean, ANOVA: Analysis of variance, CGRP: Calcitonin gene-related peptide

produced only a slight decrease at its maximal dose, in rat [31]. This result suggests that the β-adrenoceptor-mediated urethral relaxation function in male mice. Propranolol (β-blocker) has been shown to improve urinary incontinence in a small subset of patients [32].

Activation of CGRP sensory nerves was reported to elicit a remarkable urethral relaxation [33]. This study concurred with the previous report [11] that PE activated α_1-adrenoceptors of the sensory nerve and then activated capsaicin-sensitive sensory nerves to release an unknown substance that facilitated the release of PE and NE from sympathetic nerves. Subsequently, the unknown substance stimulated β-adrenoceptors in the detrusor muscle in mice, leading to neurogenic relaxation of the urinary bladder [11]. This relaxation was however inhibited by guanethidine, suggesting that the relaxation effect of PE was exerted through an enhancement of adrenergic nerve activity. In this study, the relaxation of USM was induced by nicotine, which was inhibited by lidocaine and N^G-nitro-L-arginine [Figure 5]. These results suggested that the nicotine-induced relaxation was a neurogenic response. However, the relaxation was not inhibited by guanethidine [Figure 5], suggesting that nicotine-induced neurogenic relaxation was not related to adrenergic nerve activity in the USM. These findings demonstrated that nicotine acted on nicotinic acetylcholine receptor (nAChR) that led to neurogenic relaxation in the USM. Therefore, we suggest that nAChR might locate on the CGRP sensory nerve and parasympathetic nerve (NO release). This might be similar to the chemosensory cation channel transient receptor potential A1 crucially involved in nicotine-induced irritation [26]. Nicotine was found to act in a membrane-delimited manner, stabilizing the open state(s) and destabilizing the closed state(s) of the transient receptor potential A1 channel [26].

Two limitations of the current study are lacking of molecular evidence to confirm the β-adrenoceptor expression difference between male and female urethra. In addition, the effects of adding β-adrenergic agonist alone to female urethral muscle strip are unknown.

Conclusion

β-Adrenergic receptor was prominent in female USM to facilitate urethral relaxation. The use of β-adrenergic receptor blocker agents might have the potential for the treatment of female voiding dysfunction.

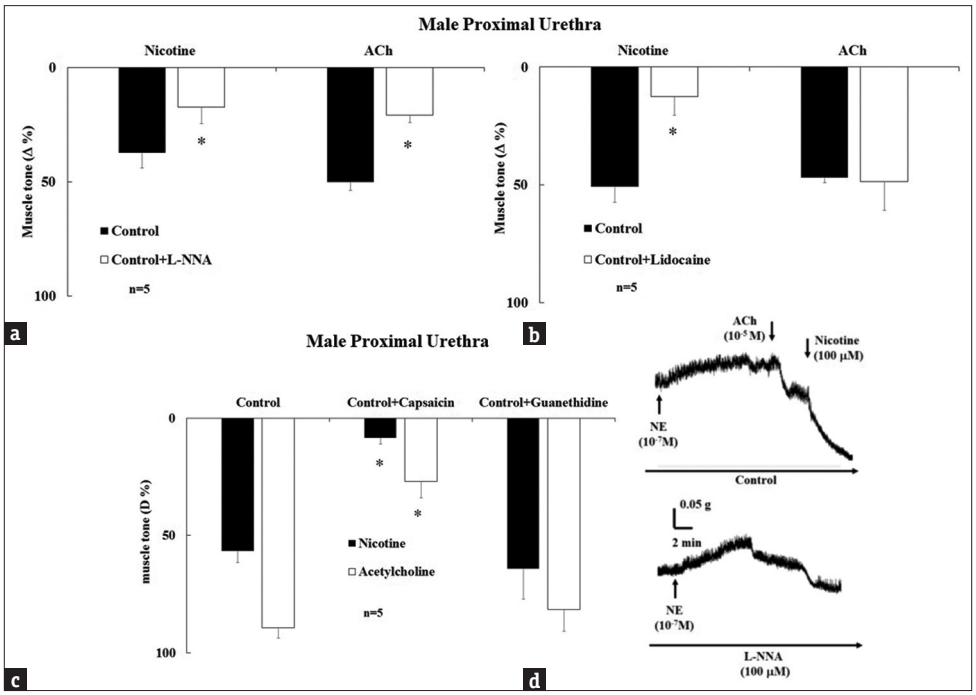

Figure 5: Effect of nitric oxide synthase inhibitor, and lidocaine, and CGRP deplete, and guanethidine on proximal USM rings relaxation in male mice. In urothelium-intact USM rings are precontracted by PE (10 μM). Relaxation response induced by nicotine (50 μM) and acetylcholine (10 μM) was inhibited by N^G-nitro-L-arginine (100 μM) (a), lidocaine (1 μM) (b), and Capsaicin (1 μM) (d). A representative tracing in (c) shows that nicotine (50 μM) and acetylcholine (10 μM) was inhibited by N^G-nitro-L-arginine (100 μM) in female proximal urethra. In contrast, this relaxation did not affect by guanethidine (10 μM) (d). n, number of experiments. Values are means ± SEM. Asterisk indicates a significant difference in Bonferroni posttests following ANOVA (*$P < 0.05$). USM: Urethral smooth muscle, PE: Phenylephrine, SEM: Standard error of the mean, ANOVA: Analysis of variance, CGRP: Calcitonin gene-related peptide

Data Availability Statements

The datasets analyzed during the current study are available from the corresponding author on reasonable request

Financial support and sponsorship

This work was supported by grants from Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (TCMF-CM1-111-04).

Conflicts of interest

There are no conflicts of interest.

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