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Original Article

The aging effects on phenylephrine-induced relaxation of bladder in mice

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Abstract

Objective: We have demonstrated that phenylephrine (PE) activates the capsaicin-sensitive nerves, and then activates capsaicin-sensitive nerves to release an unknown substance that facilitates the release of norepinephrine (NE) from adrenergic nerves. Subsequently, NE stimulates β-ARs in the detrusor muscle in mice, leading to neurogenic relaxation of the urinary bladder (UB). **Materials and Methods:** We examined if there existed sensory-motor dysfunction in UB of aging mice. To investigate the change of PE-induced detrusor relaxation in aging male-C57BL/6 mice (12- vs. 24-month-old mice), UB strips from mice were isolated, cut into strips, and mounted in the organ bath. **Results:** The UB strip contractility responding to various agents was estimated using tissue bath wire myography. Acetylcholine (ACh) and KCl-induced UB strips contraction was not significantly different between 24- and 12-month mice. NE-induced UB strips relaxation was significantly lower in 24-month than 12-month mice. Denuded bladder strips showed similar decreased relaxation response to NE. This NE-induced relaxation was inhibited by silodosin and lidocaine. PE did not induce contraction in UB strips of aging mice. In contrast, PE-induced relaxation was weaker in 24-month than 12-month mice. **Conclusion:** Our results suggested that the PE-induced relaxation was age related. Aging seemed to lead the sensory-motor dysfunction. More animal and human studies are required to prove this concept and its clinical usefulness in the future.

Keywords: *Adrenergic receptor, Aging, Bladder, Lower urinary tract dysfunction, Sensory-motor interaction*

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Introduction

It is well known that the urinary bladder (UB) is innervated both by sensory and adrenergic nerve [1], and β–Adrenoceptors activation plays an important role in the facilitation of urine storage [2,3]. With aging, norepinephrine (NE)-induced relaxation of UB was decreased [4], and increase maximum contraction elicited by both phenylephrine (PE) and NE [5-7], or NE alone [8]. This β-adrenergic relaxation dominates over α-adrenergic facilitation of bladder contractility, which is shifted toward α_1-adrenergic facilitation probably with aging in the healthy rat bladder [4]. In addition, adrenergic activity on bladder contractility increases with aging was due to overexpression of the α_{1D}-adrenoceptors [6]. However, the maximum contractions were elicited by KCl in bladder which was unaffected [5,9]. However, the α_{1A}-adrenoceptors may play an important role in an age-related increase of α_1-adrenoceptors response in UB [10].

In our previous study [11], we have demonstrated that PE activates the capsaicin-sensitive nerves to transmit unknown transmitters to act on the adrenergic nerve to release NE that acts on β-adrenoceptors to produce detrusor relaxation in 12-month-old mice. We hypothesize that the PE-induced neurogenic relaxation was an aging process. Therefore, we examined the UB to determine whether there existed sensory-motor function in aging mice.

Materials and methods

Tissue preparation

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee (105-IACUC-015, 106-IACUC-003), and performed in accordance with the guidelines of the National Institutes of Health on the care and use of laboratory animals. Animals were maintained under controlled light (12-h light/dark cycles from 7:00 AM

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to 7:00 PM) and temperature (21°C–23°C) conditions. Male C57BL/6 mice were used in all experiments. The male 12- and 24-month-old C57BL/6 mice were sacrificed by cervical dislocation after anesthesia with urethane (500 mg/kg, intraperitoneally) and chloralose (50 mg/kg, intraperitoneally). The UB was dissected (Width: 4 mm; Length: 10 mm) and placed in oxygenated (95% O_2 and 5% CO_2) Krebs solution at 4°C. The composition of the Krebs bicarbonate solution (in mM) was 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 (EDTA) 0.023.

Tissue-bath wire myography for the urinary bladder

The UBs were dissected and cleaned of surrounding tissue under a dissecting microscope, and then mounted on a stainless steel rod and a platinum wire in a tissue bath containing 20 mL of Krebs solution that was equilibrated with 95% O_2 and 5% CO_2 and maintained at 37°C. The mucosa was removed by tissue forceps to form urothelium-denuded bladder strips. Tension changes were measured by an isometric transducer (FT03C; Grass) and recorded on a Powerlab polygraph (LabChart 7, v7.1. ADInstruments Pty Ltd., Castle Hill, Australia). These UB strips were equilibrated in the Krebs solution for 60 min and mechanically stretched to a resting tension of 5 mN Step 1: After equilibration, the resting muscle tone of the UB strips was changed by cumulative applications of PE (0.001–10 μM). Step 2: After washing, the resting muscle tone of the UB strips was changed by cumulative applications of Acetylcholine (Ach) (0.001–10 μM). Between Steps 1 and 2, the UB strips were washed for 45 min with Krebs solution. Step 3: After the washes were completed, silodosin (0.1 μM) or lidocaine (0.1 mM) was added 15 min before PE administration (0.001–10 μM), after which the relaxation effects were recorded. Step 4: Maximal contraction of the UB strip was induced by KCl (70 mM). Only 1 isolated UB strip per animal was used in the myography study. Changes in muscle contraction tone were estimated as percentages of the KCl (70 mM)-induced maximum contraction.

Drugs used and statistical analysis

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

Results

The KCl- and acetylcholine-induced contraction in the urinary bladder of aging mice

The maximal UB contraction was obtained by high concentration (70 mM) of KCl in bladder strips with urothelium (12 months: 337.8% ± 58.1%, $n = 11$; 24 months: 395.7% ± 112.7%, $n = 8$). No age-related changes were observed in 70 mM KCl-induced contractions [Figure 1a]. The concentration-response curves for ACh [0.001–10 μM, Figure 1b] in UB strips with urothelium were depicted as the percentage of maximal response to KCl (% KCl-induced contraction). The ACh-induced contractions were not different in between 12- and 24-month mice [$n = 11$~6, $P < 0.05$, Figure 1c].

The norepinephrine-induced relaxation in bladder strips of aging mice

In the presence of active muscle tone induced by ACh (10 μM), the bladder strips with [Figure 2a] or without [Figure 2b] mucosa relaxed on the application of NE (0.001–10 μM) in aging mice. The NE-induced relaxation was significantly lower in 24-month mice than 12-month mice ($n = 6$, $P < 0.05$) in both UBs. This NE-induced relaxation

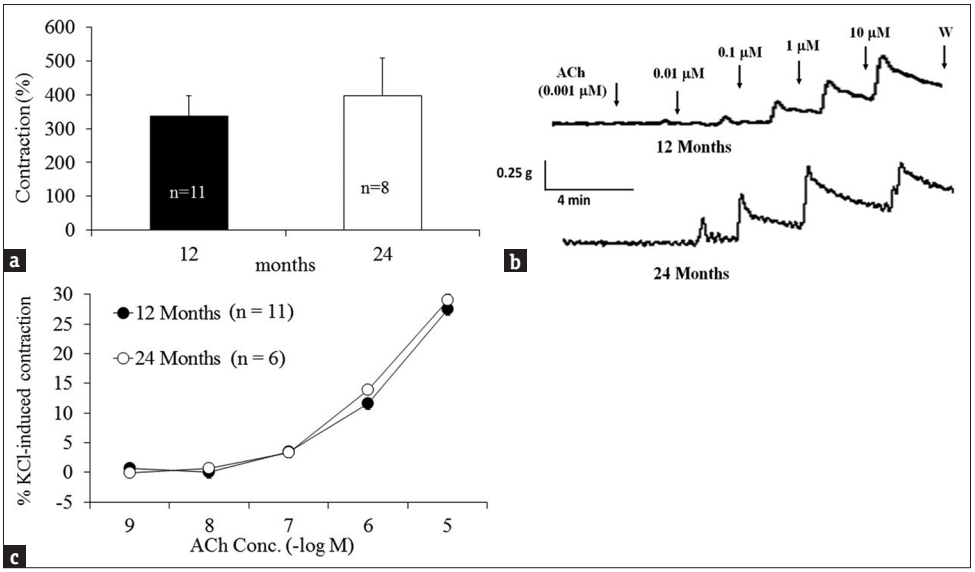

Figure 1: Effects of age on KCl- and acetylcholine-induced contraction. The KCl (70 mM)-induced maximal contraction in 12- and 24-month mice were not significantly different ($n = 11$-8, $P > 0.05$, a). A representative tracing shows acetylcholine (acetylcholine, 0.001–10 μM)-induced contraction in 12-month (upper panel, b), and 24-month (lower panel, b) mice, in urothelium intact urinary bladder in the absence of active muscle tone. This constriction was not different between 12- and 24-month bladder strips ($n = 11 \sim 6$, $P > 0.05$, c). Values are mean ± standard error mean, n = number of experiments. W: Wash

was inhibited by lidocaine (0.1 mM) and silodosin (0.1 μM) in 12-month mice [Figure 2c, $n = 5$, $P < 0.05$].

The phenylephrine-induced relaxation in bladder strips of aging mice

In the absence of active muscle tone, the UB strips with [Figure 3a] or without [Figure 3b] mucosa relaxed on the application of PE (0.001–10 μM) in aging mice. In 24-month mice, the PE-induced relaxation was significantly lower than 12-month mice ($n = 5$, $P < 0.05$). The results were summarized in Table 1.

Discussion

We demonstrated that the PE-induced relaxation of bladder strips appeared in both 12-month-old mice and 24-month-old mice. On dose-response test, ACh and KCl-induced UB strips contraction was not significantly different between 24- and 12-month mice. Therefore, the PE-induced neurogenic relaxation mechanism of UB might be an aging process [Figure 3].

The sensitivity of bladder contraction to muscarinic receptor agonist was increased in aged rats [12]. However, in the isolated whole bladder model, the contractile response to the bethanechol (a muscarinic receptor agonist) did not change significantly with age [9]. However, in our tissue bath study, the ACh-induced contractions were significantly lower in 24-month-old mice than 12-month mice in the bladder with urothelium, while the ACh-induced contractions were not changed in mice without endothelium. These results suggested that bladder mucosa may involve in the aging process of motor signaling and ACh-induced contraction [Figure 1].

The maximum contractions of UB were elicited by KCl, and such contraction was unaffected in aged mouse [5]. In the present study, the KCl-elicited maximum contractions had no significant difference in both groups. KCl-induced contraction has been known to be due to membrane depolarization causing Ca^{2+} entry through voltage-operated Ca^{2+} channels, activation of Ca^{2+}-dependent myosin light chain (MLC) kinase, and increases in MLC phosphorylation in smooth muscle cell [4]. Therefore, equal maximal bladder strips contraction response

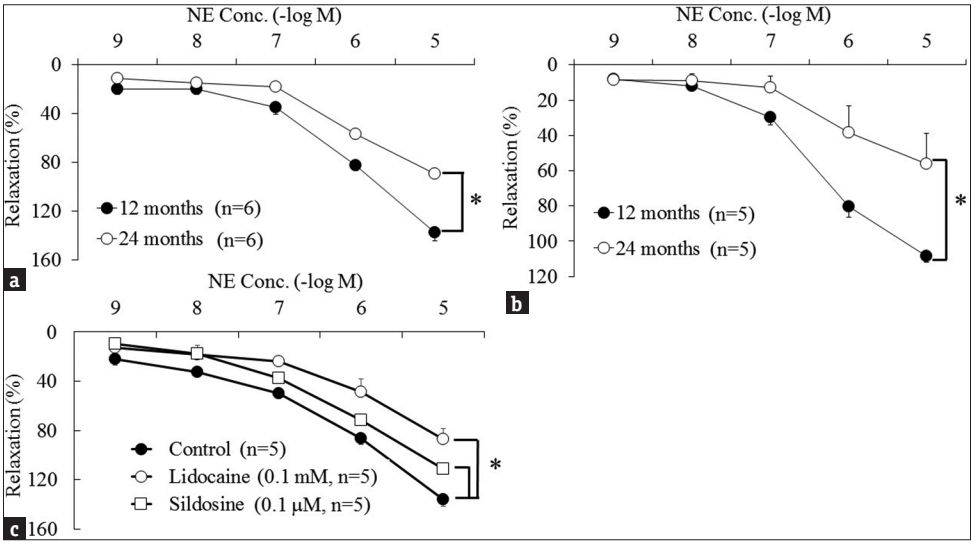

Figure 2: Effects of age on norepinephrine-induced relaxation. Norepinephrine (NE, 0.001–10 μM)-induced relaxation in with (a) and without (b) mucosa bladder strips of aging mice (12 and 24 months, $n = 6$), in the presence of active muscle tone induced by acetylcholine (10 μM). The NE-induced relaxation was significantly greater in 12-month than that in 24-month mice urinary bladder ($n = 6$, $P < 0.05$). This NE (0.001–10 μM)-induced relaxation was significantly inhibited by silodosin (10 μM, $n = 5$, c), and lidocaine (0.1 mM, $n = 5$, $P < 0.05$, c) in 12-month mice with urothelium. Values are mean ± standard error mean. n = number of experiments. W: wash. Asterisk indicates a significant difference in Bonferroni posttests following ANOVA (*$P < 0.05$)

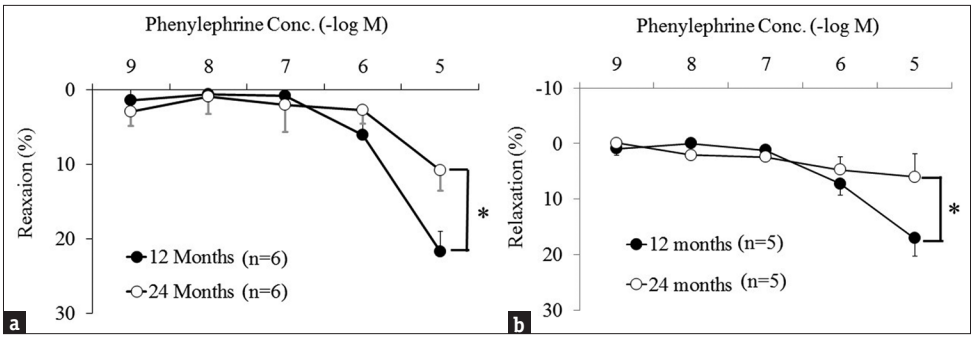

Figure 3: Effects of age on phenylephrine-induced relaxation. The phenylephrine (PE, 0.001 ~ 10 μM)-induced relaxation was significantly greater in 12-month (a, $n = 6$) mice than that in 24-month (a, $n = 6$) mice urinary bladder ($P < 0.05$). A similar result was found in without mucosa UB of aging mice (b, $n = 5$, $P < 0.05$). Values are mean ± standard error mean. n = number of experiments. Asterisk indicates a significant difference in Bonferroni posttests following ANOVA (*$P < 0.05$)

Table 1: Summary of maximal contraction and relaxation between the 12- and 24-month mice urinary bladder strips (mean ± standard error of mean)

Age (months)	12 months (change of length, percentage)		24 months (change of length, percentage)		*P*
	Urothelium (with)	Urotheliumb (denuded)	Urothelium (with)	Urothelium (denuded)	
KCl (contraction, 70 mM)	337.8 ± 58.1 (*n*=11)	329.3 ± 36.1 (*n*=6)	395.7 ± 112.7 (*n*=8)	445.1 ± 120.1 (*n*=6)	0.473
ACh (contraction, 10 μM)	27.4 ± 2.1 (*n*=11)	38.9 ± 0.8 (*n*=6)	29.8 ± 1.1 (*n*=6)	36.7 ± 2.4 (*n*=6)	0.633
NE (relaxation, 10 μM)	137.6 ± 5.6 (*n*=6)	108.4 ± 1.2 (*n*=5)	85.8 ± 1.6 (*n*=6)	56.2 ± 10.5 (*n*=5)	<0.001
PE (relaxation, 10 μM)	21.7 ± 2.7 (*n*=6)	23 ± 1.8 (*n*=5)	10.8 ± 3.1 (*n*=6)	6.7 ± 1.5 (*n*=5)	<0.01

P value was compared to 12 months mice. ACh: Acetylcholine, NE: Norepinephrine, PE: Phenylephrine, KCl: Potassium chloride

may be an alternative way to set an equal amount of contractile units in each group.

Decreases in β-adrenergic-induced relaxation response were noted with age in rat bladder [4], and there might be one of the causative factors of reduced bladder compliance in the elderly [13]. In contrast, it has reported that no age-related changes were observed in isoproterenol-induced relaxation of the bladder [12]. In the present study, the relaxation of bladder with- or without-endothelium was induced by NE. This relaxation was significantly lower in 24-month mice than 12-month mice. These results suggested the NE-induced relaxation decreased in aged mice. Immunostaining for identification of receptor density might be an alternative way to evaluate the aging process on mice UB.

In the present study, the PE-induced relaxation of bladder strips appeared in 12-month-old mice, and there was significantly decreased in 24-month-old mice. Previous studies suggested that aging is associated with increasing neurogenic enhancement of bladder filling compliance [14], while no change in detrusor power or contractile force during voiding detrusor contractility during bladder voiding [15].

Conclusion

This is the first study demonstrating the PE-induced relaxation was age-related alterations. More animal and human studies are required to prove this concept and its clinical usefulness in the future.

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Conflicts of interest

There are no conflicts of interest.

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