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Differences between young and aged rats in voiding frequency and detrusor muscle serotonergic contraction

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ABSTRACT

Introduction: The involvement of serotonin (5-HT) in increased lower urinary tract symptoms in aging is unclear. We sought to compare voiding function and 5-HT induced detrusor contraction between young and aged rats.

Methods: This study used young (2- to 3-month-old) and aged (26- to 30-month-old) male Fischer 344 rats. 1. Rats were housed in individual metabolic cages, and then the total volume of urination, volume per micturition, voiding frequency, and voiding interval were analyzed. 2. Using urinary bladder body strips, developed tension was recorded after cumulative addition of 5-HT (1-100 nM) in the absence or presence of tetrodotoxin (1 μ M), and in the presence of tetrodotoxin with ketanserin (0.3–3 μ M) or naftopidil (1 and 3 μ M). We examined the effects of atropine, ketanserin, and naftopidil on electrical field stimulation (EFS)-induced contraction.

Results: 1. Compared to young rats, aged rats exhibited decreased voiding frequency and increased volume per micturition, but total volume of urination (normalized to body weight) did not differ. Moreover, voiding interval was significantly prolonged in aged rats during the active period. 2. In the presence of tetrodotoxin, pEC₅₀ of 5-HT were significantly lower in aged rats than in young rats (P < 0.01), but the maximal response to 5-HT was not altered in the aged bladder. Ketanserin inhibited 5-HT-induced contraction in both groups, while suppression by naftopidil was relatively limited, especially in aged rats. EFS induced neurogenic contraction in a frequency-dependent manner. Atropine-resistant contraction was not inhibited by naftopidil, but was potentiated by ketanserin.

Conclusions: Urination intervals were extended in aged rats, indicating that urination rhythm changed. In the senescent rat bladder, 5-HT induced detrusor contraction, but the effect of 5-HT and the naftopidil-sensitive contractile force were weaker than those in young rats. Additionally, 5-HT did not contribute to the increase in atropine-resistant EFS-induced contractions in aged rats.

Keywords: aged rats, voiding function, ketanserin, naftopidil, serotonin, urinary bladder

1. INTRODUCTION

Aging is associated with an increase in lower urinary tract symptoms, such as urinary frequency (daytime frequency), incontinence, and voiding dysfunction with residual urine (incomplete emptying), in addition with decreased bladder contraction (underactive bladder) and uninhibited bladder contraction (overactive bladder) (Okamura et al., 2002; Pfisterer et al., 2006). These symptoms affect the quality of life of aged persons (Homma et al., 2006). Although the precise mechanisms underlying age-related bladder dysfunction and increased lower urinary tract symptoms are unclear, it is known that the prevalence of urinary tract dysfunction generally increases with age.

In the voiding phase, parasympathetic (cholinergic) nerves play a main role, as released acetylcholine activates smooth muscle of the bladder body by activating muscarinic M3 receptors, leading to voiding (Andersson and Arner, 2004; Andersson and Wein., 2004). Therefore, the focus of pharmacological therapy for lower urinary tract symptoms has been on muscarinic receptor antagonists. However, muscarinic receptor antagonists often have unsatisfactory effects on improving symptoms in elderly people. For example, lower urinary tract symptoms from bladder overactivity have been considered to be due, at least in part, to increased contraction induced through a pathway other than that for muscarinic receptor activation by acetylcholine. Among the candidate transmitters that have been examined to date, purinergic contraction by adenosine triphosphate (ATP), α -adrenergic contraction, and serotonergic contraction by 5-hydroxytryptamine (5-HT; serotonin) are possible factors underlying age-related bladder dysfunction (Saito et al. 1993; de Groat et al., 2015; Silva et al., 2015).

Among these neurotransmitters, 5-HT may play a role in controlling micturition through various mechanisms, including central pathways (Ramage, 2006). Serotonin has several pharmacologically distinct receptors. 5-HT₂ receptors, which have three subtypes (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}), have multiple roles in various organs and tissues (e.g., contracting blood vessels and modulating the secretion of hormones), and 5-HT₂ receptors are associated with memory formation and psychological disorders, including depression (Nagatomo et al., 2004; Kaye et al., 2005; Lohoff et al., 2010; Ebdrup et al., 2011; Lohoff et al., 2013; Gibbs and Hertz, 2014). There are many reports on the physiological roles of 5-HT in different species. The urinary bladder is contracted by 5-HT through the 5-HT₃ receptor in rabbits

(Chen, 1990), whereas 5-HT induces bladder contraction through 5-HT1A, 5-HT2A, and 5-HT2B receptors in rats (Sakai et al., 2013a), and through the 5-HT4 receptor in humans (Chapple, 2004). Therefore, it is likely that there is a wide range of differences among species in 5-HT-induced smooth muscle contraction of the urinary bladder. Furthermore, contraction induced by 5-HT is augmented in rats with bladder outlet obstruction (BOO) (Sakai et al., 2013b), which is an overactive bladder model. One report suggested that the increased contractile responses to 5-HT in rats with BOO may be caused, in part, by 5-HT2A receptor upregulation in detrusor smooth muscles (Sakai et al., 2013b).

In the present study, we sought to compare voiding function and 5-HT induced detrusor contraction between young and aged rats. We hypothesized that the frequency of micturition and contraction induced by 5-HT are altered in aged rats. First, we investigated whether the voiding function is deteriorated in aged rats. Second, we examined whether 5-HT-induced bladder smooth muscle contraction is altered in aged rats, and whether the inhibitory effect of the 5-HT2 receptor antagonist on this contraction changes. Third, we examined the effects of the 5-HT2 receptor antagonist on electrical field stimulation (EFS)-induced neurogenic contraction to determine the involvement of 5-HT receptor-mediated contraction in atropine-resistant contraction, and the possible role of 5-HT in increased lower urinary tract symptoms in aged bladder.

2. METHODS

All experiments were performed in accordance with the *Guide for Care and Use of Laboratory Animals* published by the United States National Institutes of Health (NIH publication nos. 85–23, revised in 1996) and the *Regulations of Animal Experiments at Yamagata University*, under the regulation of the Animal Care Committees of Yamagata University School of Medicine (identification no.: 25072) and Juntendo University (identification no.: 1205).

2.1. Materials

Male young (2- to 3-month-old) and aged (26- to 30-month-old) Fischer 344 rats were obtained from Charles River Japan (Atsugi, Japan).

Atropine sulfate monohydrate was purchased from Wako Pure Chemicals (Osaka, Japan). Serotonin (5-hydroxytryptamine hydrochloride; 5-HT), ketanserin, and tetrodotoxin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Naftopidil ((±)-1-[4-(2-methoxyphenyl) piperazinyl]-3-(1-naphthyloxy) propan-2-ol) was synthesized in Asahi Kasei Pharma Corporation (Tokyo, Japan). Naftopidil was dissolved in 0.1 M phosphate buffer, and ketanserin was dissolved in dimethyl sulfoxide (DMSO, final concentration ≤ 0.03%) and diluted using distilled water, respectively.

2.2. Voiding Function

Rats (young, n = 6; aged, n = 12) were housed in individual metabolic cages and provided with free access to water and pellet food during the experimental periods. Rats were maintained under a light (5:00 a.m.-7:00 p.m.) and dark (7:00 p.m.-5:00 a.m.) cycle.

After 48 hours of acclimation of each rat to its cage, an electronic balance (FZ-300iW, AND, Tokyo, Japan) was placed under the metabolic cage to measure urine volume. The urine volume was measured every 30 seconds over the subsequent 48 hours. The total volume of urination, volume per micturition, voiding frequency, and voiding interval were analyzed using a personal computer and electronic balance data (Ramage, 2006). Water and pellet intake were measured.

2.3. 5-HT-induced Contraction

Rats (young, n = 6; aged, n = 9) were sacrificed under anesthesia with pentobarbital (30 mg/kg, ip). Urinary bladders were excised, and excess fat and connective tissues were removed carefully. The urinary bladder was cut into four strips with mucosa from the bladder body. Each bladder strip (10 × 1.5 mm) was suspended in an organ bath (37 ± 0.1°C) containing 10 mL modified Krebs–Henseleit solution. This solution contained 118 mM NaCl, 4.7 mM KCl, 24.9 mM NaHCO₃, 1.18 mM MgSO₄, 1.18 mM KH₂PO₄, 11.1 mM glucose, and 1.8 mM CaCl₂. A highly concentrated K⁺ solution was made by substituting NaCl with equimolar KCl. These solutions were adjusted to pH 7.4 and saturated with 95% O₂ and 5% CO₂ at 37°C. The developed tension was recorded using an isometric force transducer (7T15-240, Orientec, Tokyo, Japan) for measurement of changes in the contractile force. The preparation was stretched to a resting tension of 1.0 g, and

the solution was changed every 15 min. After an equilibration period of 1 h, each preparation was contracted with 66.7 mM KCl (high K⁺) repeatedly until reproducible contraction was attained. To study 5-HT receptor-mediated contraction, 5-HT (10⁻⁹–10⁻⁴ M) was cumulatively added in the absence or presence of tetrodotoxin (1 μM). Subsequent experiments were carried out in the presence of tetrodotoxin (1 μM) to inhibit possible spontaneous nerve-mediated contractions. Incubation for 20 min with ketanserin, a 5-HT_{2A} receptor antagonist, and naftopidil, a 5-HT_{2A/2B} antagonist, was performed before addition of 5-HT.

2.4. EFS-induced Contraction

To investigate the effect of 5-HT₂ receptor antagonists on neurogenic contraction of bladder smooth muscle from young (n = 6) and aged rats (n = 9), each fresh detrusor muscle strip (10 × 1.5 mm) was suspended between platinum ring electrodes in an organ bath. EFS was applied using a stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan) as follows: trains of 3 s at 1–128 Hz, supramaximal voltage (40 V), 0.2-ms pulse duration, applied every 3 min. To ensure that the EFS-induced contraction was neurogenic, tetrodotoxin was added and shown to completely inhibit EFS-induced contraction. After the first frequency-dependent response was examined, each strip was incubated with the muscarinic receptor antagonist atropine for 20 min, followed by the second EFS, which was applied in the presence of atropine. When EFS-induced contraction was obtained in the presence of atropine (1 μM), the event was considered an atropine-resistant contraction. Ketanserin (3 μM) or naftopidil (3 μM) was then added and strips were incubated for 20 min before EFS. The third EFS was then carried out in the presence of atropine and ketanserin or naftopidil to investigate the effects of these compounds on atropine-resistant contraction.

2.5. Statistical Analysis

All data are expressed as means ± standard errors of the mean. Parameters of voiding function were compared as raw values and as body weight-normalized ratios, given that there was a weight difference between young and aged rats.

5-HT and EFS-induced contraction data were expressed as the percentage of maximal response to KCl (66.7 mM).

Differences between young and aged rats in vivo were analyzed using the unpaired Student's t-test. Each CRC for 5-HT in the presence and absence of tetrodotoxin (TTX) was compared to the corresponding CRC between the young and aged rats, or in the presence of ketanserin and naftopidil to the corresponding vehicle CRC, using F-test. Then, values of the negative logarithm of the concentration required for a half maximal response to 5-HT (pEC_{50}) and the maximal effect induced by 5-HT (E_{max}) were summarized. The comparison of whole curves was conducted first. When statistical significance was found, pEC_{50} and E_{max} were compared using the Tukey post-hoc test. In addition, CRCs in the presence of ketanserin and naftopidil were assessed for parallelism. When the results of CRC analysis were statistically significant, an analysis for parallelism was performed. The value of pA_2 is calculated by Schild plot after acceptance of parallelism. All analyses were performed as two-tailed tests. Statistical analyses were performed using JMP® 14 (SAS Institute Inc., Cary, NC, USA) and Kaleida Graph 4.1 (Synergy Software, Reading, PA, USA). Differences were considered statistically significant when P was < 0.05 .

3. RESULTS

3.1. Voiding Function

3.1.1. Water intake

The daily amount of water intake was lower in aged rats than in young rats. When expressed as a percentage of body weight, the water intake over 24 hours was significantly decreased ($P < 0.001$) in aged rats compared to young rats (Table 1).

3.1.2. Urine volume

The volume per micturition was significantly increased in aged rats compared to young rats. However, when measured as the average total volume of urination over 24 hours, there were no significant differences in body weight-normalized ratios between aged rats and young rats (Table 1).

Table 1: Comparison of parameters between young and aged rats

	Young (n=6)	Aged (n=12)
Body weight (g)	212.57 ± 2.46	362.68 ± 10.62 **
Water intake		
Weight (g)	21.25 ± 0.67	15.03 ± 1.71
Ratio (%)	10.01 ± 0.32	4.12 ± 0.47 **
Volume per micturition		
Weight (g)	0.46 ± 0.01	1.06 ± 0.12 *
Ratio (%)	0.22 ± 0.01	0.29 ± 0.03 *
Total volume of urination over 24 hours		
Weight (g)	5.23 ± 0.29	8.01 ± 0.80
Ratio (%)	2.46 ± 0.13	2.23 ± 0.27

% of body weight ratio, data are expressed as means ± SEM. *P < 0.05, **P < 0.01 vs. Young by the unpaired Student's t-test.

3.1.3. Micturition frequency

The micturition frequency was significantly lower (P < 0.01) in aged rats (range, 3-15 times/day) than in young rats (range, 8-13 times/day) (Table 2).

3.1.4. Micturition interval

Both groups exhibited longer intervals of micturition during the light (rest) periods than during dark (active) periods. Intervals of urination in the dark period were significantly longer in aged rats than in young rats (Table 2).

Table 2: Average micturition frequency and voiding interval during light and shade periods

	Young (n=6)	Aged (n=12)
Average micturition frequency (times/day)	11.08 ± 0.43	8.50 ± 0.60 **
Average voiding interval (min)		
Dark (active) period	103 ± 5	131 ± 7 §
Light (rest) period	158 ± 9	187 ± 12

Data are expressed as means ± SEM. **P < 0.01, §P < 0.002 vs. Young by the unpaired Student's t-test.

3.2. Contraction Induced by KCl in the Young and Aged Bladder

KCl-induced contraction was significantly greater in the young bladder than in the aged bladder when the tissue weight was taken into account ($P < 0.01$). Bladder body strips in young and aged rats were almost the same in length, but the width and weight in aged rats (1.79 ± 0.06 mm and 21.06 ± 0.91 mg, respectively; $n = 9$) were significantly greater than those in young rats (1.16 ± 0.06 mm and 10.56 ± 0.59 mg, respectively; $n = 6$, $P < 0.0001$). Therefore, KCl-induced contraction normalized by strip length and weight was significantly lower in aged rats than in young rats (Fig. 1; $P < 0.0001$).

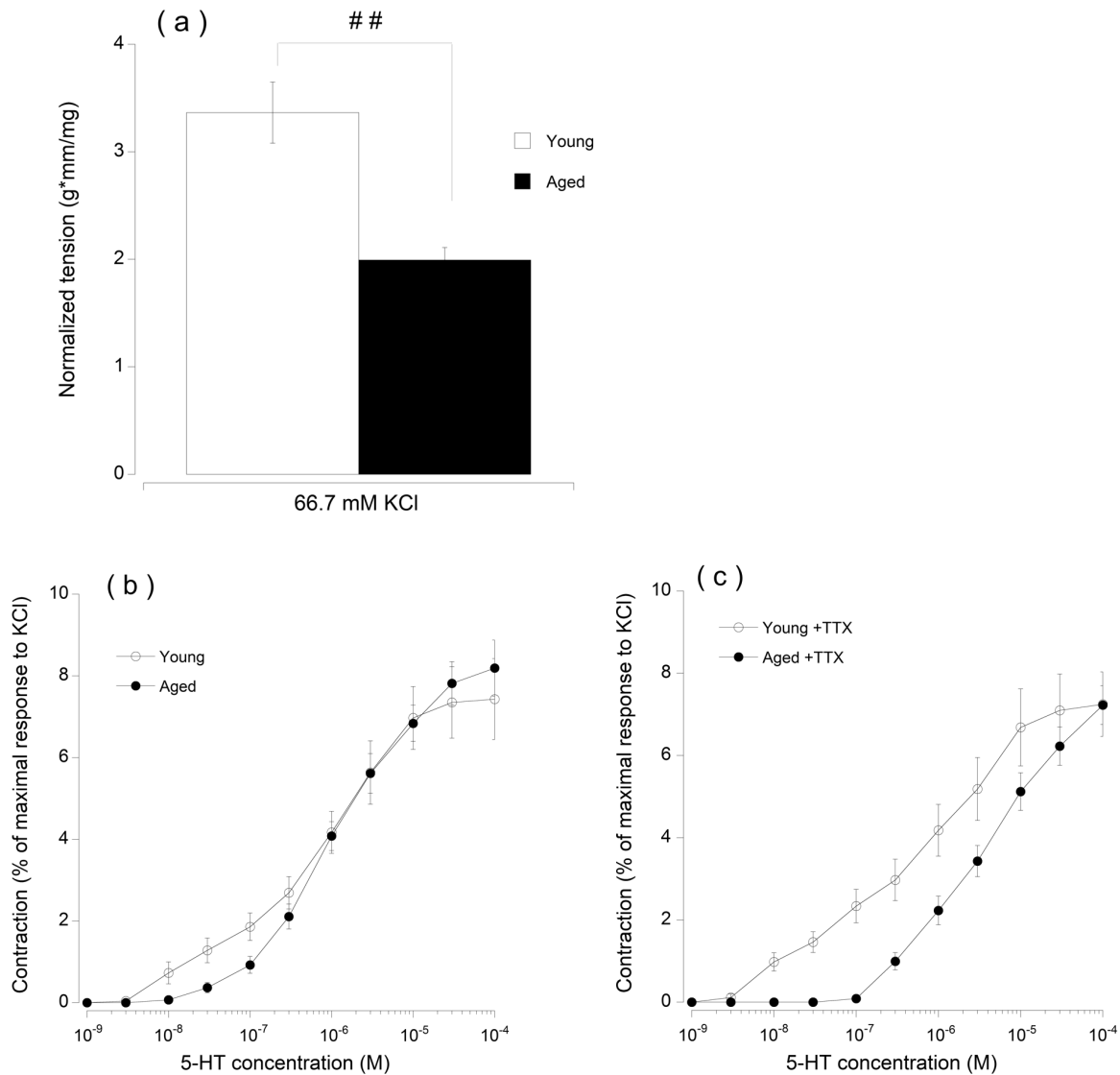


Fig. 1 Contractions induced by KCl and 5-HT in bladder strips from young and aged rats.

Isolated bladder strips were mounted in organ baths containing 10 mL modified Krebs-Henseleit solution, then KCl-induced contraction was measured by replacement with 66.7 mM KCl solution **(a)** or 5-HT-stimulated contraction was measured. 5-HT was added cumulatively (10^{-9} to 10^{-4} M) in the absence **(b)** or presence **(c)** of tetrodotoxin ($1 \mu\text{M}$). Data were normalized by strip length and weight, and expressed as mean \pm SEM of samples from young ($n = 6$) and aged ($n = 9$) rats. ##P < 0.01 vs. young rats by the unpaired Student's t-test.

3.3. Contraction Induced by 5-HT in the Young and Aged Bladder

The force of contraction was increased by 5-HT in a concentration-dependent manner in the isolated bladder strips of young and aged rats. The minimum concentration which induces contraction by 5-HT was higher in aged rats than in young rats, although the maximal response to 5-HT did not differ (Fig. 1b and c). CRCs for 5-HT were equivalent between the young and aged groups in the absence of TTX ($P = 0.6066$) (Fig. 1b); however, they were significant in the presence of TTX ($P = 0.0420$) (Fig. 1c). pEC_{50} was lower in the aged group than in the young group in the presence of TTX and in the aged group in the absence of TTX (Table 3). The effects of ketanserin on CRCs for 5-HT were determined in both the young and aged rats ($P = 0.0138$ and 0.0008 , respectively) (Figs. 2a, b). The value of E_{max} was significantly lower after administration of 1×10^{-6} and 3×10^{-6} M ketanserin ($P < 0.05$ each for young and aged preparations) (Table 4). Parallelism for the CRCs in the presence of ketanserin was rejected significantly in both the young and aged groups ($P = 0.0022$ and 0.0088 , respectively), thereby indicating non-competitive antagonism of ketanserin. Although the minimum concentrations of 5-HT needed to induce contraction were respectively 10^{-8} , 3×10^{-9} , 3×10^{-8} and 10^{-7} M in the young preparations treated with control or ketanserin ($0.3-3 \mu\text{M}$) (Fig. 3a), the concentrations were 10^{-7} , 10^{-7} , 10^{-7} and 10^{-6} M, respectively, in the aged preparations (Fig. 3b). The concentration-dependent response to ketanserin disappeared at less than 3×10^{-6} M 5-HT in the aged preparations. In the presence of naftopidil, CRCs for 5-HT were equivalent between the vehicle (distilled water) and naftopidil groups in both the young and aged rats ($P = 0.2890$ and 0.2891 , respectively). Therefore, the values of pA_2 were not calculated. The results for the concentration-response parameters for naftopidil are summarized in Table 4. Although the minimum concentrations of 5-HT needed to induce contraction were respectively 10^{-8} , 3×10^{-9} and 10^{-7} M in the young preparations treated with control or naftopidil (1 and $3 \mu\text{M}$) (Fig. 3c), the concentrations were all 3×10^{-7} M in the aged preparations (Fig. 3d). The concentration-dependent response to naftopidil disappeared entirely in the aged preparations.

Table 3: Concentration response parameters for 5-HT between the young and aged groups.

	(-) TTX		(+) TTX	
	Young	Aged	Young	Aged
pEC ₅₀	6.0 ± 0.1	5.9 ± 0.1	6.2 ± 0.2	5.3 ± 0.1 ^{**##}
E _{max}	8.7 ± 1.2	8.9 ± 0.8	8.9 ± 0.9	8.1 ± 0.6
n	6	9	6	9

E_{max}, the maximal effect induced by 5-HT; pEC₅₀, the negative logarithm of the concentration required for a half maximal response to 5-HT; TTX, tetrodotoxin. ^{**} P < 0.01 vs. the young group in the presence of TTX; ^{##} P < 0.01 vs. the aged group in the absence of TTX.

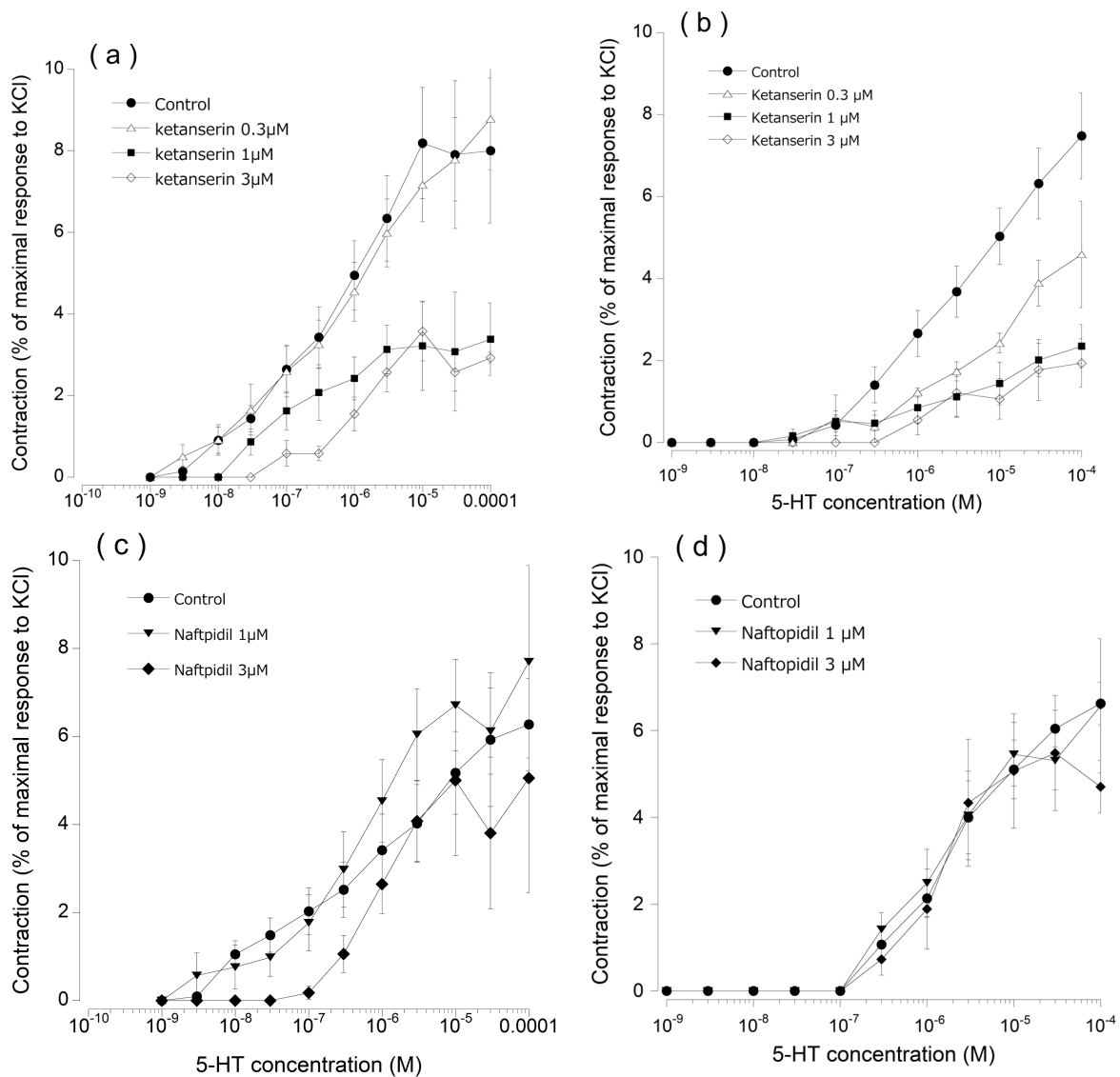


Fig. 2 Effects of the 5-HT₂ receptor antagonist ketanserin (**a** and **b**) and naftopidil (**c** and **d**) on 5-HT-induced detrusor muscle contraction in young (**a** and **c**) and aged (**b** and **d**) bladders. Treatment with the antagonist was administered starting from 20 min before (and continuing during) 5-HT stimulation. These experiments were carried out in the presence of tetrodotoxin (1 μ M). Data are expressed as mean \pm SEM of young (n = 4-6) and aged (n = 4-9) rats.

Table 4: Concentration response parameters of ketanserin and naftopidil

	Ketanserin (M)				Naftopidil (M)		
	Vehicle	3×10^{-7}	1×10^{-6}	3×10^{-6}	Vehicle	1×10^{-6}	3×10^{-6}
<i>Young</i>							
pEC ₅₀	6.3 \pm 0.2	5.9 \pm 0.4	6.9 \pm 0.4	6.1 \pm 0.3	5.8 \pm 0.7	6.3 \pm 0.3	6.1 \pm 0.2
E _{max}	8.9 \pm 0.9	9.8 \pm 1.6	3.3 \pm 0.6*	3.1 \pm 0.6*	7.4 \pm 1.9	7.4 \pm 1.0	4.7 \pm 0.7*
n	6	6	4	4	6	5	4
<i>Aged</i>							
pEC ₅₀	5.5 \pm 0.1	5.8 \pm 0.4	5.3 \pm 0.5	5.9 \pm 0.5	5.7 \pm 0.2	5.8 \pm 0.2	5.9 \pm 0.1
E _{max}	7.3 \pm 0.6	6.7 \pm 4.6	1.9 \pm 0.8*	2.0 \pm 0.5*	6.7 \pm 0.7	6.3 \pm 0.6	5.2 \pm 0.4*
n	9	4	9	6	9	9	9

E_{max}, the maximal effect induced by 5-HT; pEC₅₀, the negative logarithm of the concentration required for a half maximal response to 5-HT. *P < 0.05 vs. the vehicle group.

3.4. Effects of Ketanserin and Naftopidil on Atropine-resistant EFS-induced Contraction

EFS induced neurogenic contraction in a frequency-dependent manner (1-128 Hz), with no difference in contractile responses between young and aged rats. In the presence of atropine, EFS-induced contraction was suppressed to nearly 50% and 60% of the maximal response to KCl in young and aged rats, respectively.

Atropine-resistant contraction tended to be stronger in aged preparations than in young preparations, but not significantly. With atropine (1 μ M), the frequency-response curve in aged preparations indicated over 80% at 20 Hz or more (Figs. 3b and d), however it indicated under 80% in young preparations (Figs. 3a and c).

Atropine-resistant contraction was not inhibited by naftopidil (3 μ M) in young and aged rats (Figs. 3a and b). Atropine-resistant EFS-induced contraction appeared

to be greater in the presence than in the absence of ketanserin in both groups, but this difference was not significant (Figs. 3c and d).

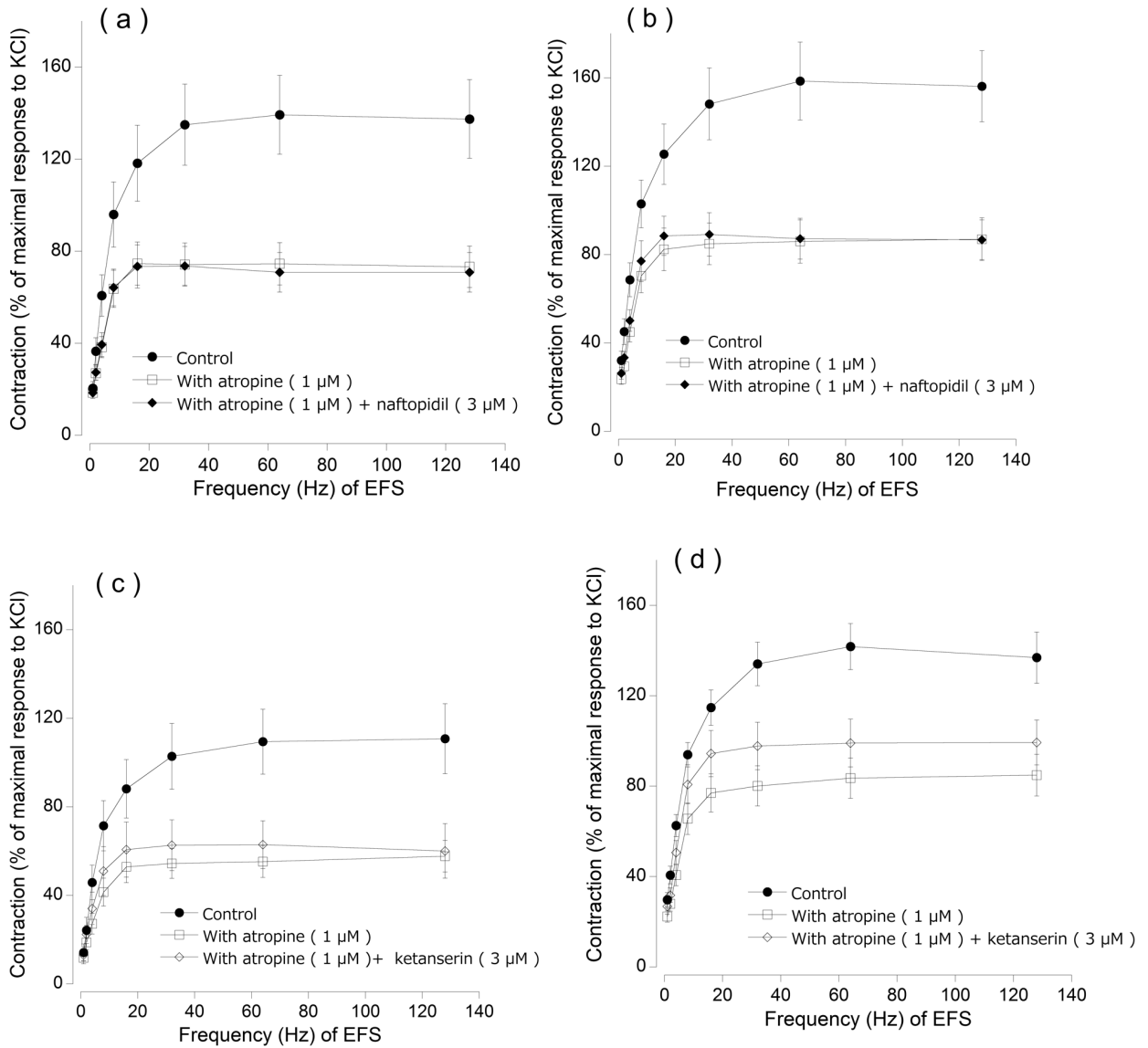


Fig. 3 Effects of naftopidil (3 μM, **a** and **b**) and ketanserin (3 μM, **c** and **d**) on atropine-resistant contraction of electrical field stimulation (EFS)-induced contraction in young (**a** and **c**) and aged (**b** and **d**) rats. Each detrusor strip was subjected to EFS, and the contraction was measured. Data are expressed as mean ± SEM of young (n = 6) and aged (n = 7) rats.

4. DISCUSSION

The main findings of the present study were that micturition frequency significantly decreased in aged rats compared to young rats. The minimum concentration of 5-HT needed to induce contraction was significantly higher in the bladder of aged rats compared to that of young rats, but maximal 5-HT-induced contraction did not differ significantly from that in young rats when expressed as the percentage of maximal response to KCl. The 5-HT-induced contraction was significantly weaker in the presence of tetrodotoxin than in the absence of tetrodotoxin only in the aged bladder. The effects of ketanserin on CRCs for 5-HT were found but not naltropidil. Additionally, 5-HT did not contribute to increased atropine-resistant contraction in response to EFS in the aged rat bladder. These results contrast with our hypothesis that 5-HT-induced contraction, mediated by 5-HT₂ receptors, participates in atropine-resistant contractions, a pattern that is known to increase with aging.

4.1 Voiding Function

The present study found that the amount of body weight-normalized water intake was significantly lower in aged rats than in young rats. However, there were no significant differences in the body weight-normalized total urine volume when comparing between the two groups. It is thought that the insensible transpiration amount associated with locomotor activity or basal metabolism is higher in young rats than in aged rats. In contrast, the volume per micturition increased in aged rats. In this regard, the results of the present study are consistent with those of a previous study that reported that the volume per micturition increased with age (Chun et al., 1988; Longhurst et al., 1992). However, a separate previous report indicated that the volume per micturition and total urine volume did not increase with age (Ito et al., 2015). It is possible that the various studies differed regarding details of living conditions or measurement procedures (e.g., in terms of adaptation period or measurement instrument used), thereby influencing the results.

In addition, voiding frequency was significantly lower in aged rats than in young rats. In this regard, the results of the present study were consistent with those of a previous report (Longhurst et al., 1992), while disagreeing with the data obtained in other previous reports indicating that the voiding frequency increased (Chun et al., 1988) or was unchanged (Ito et al., 2015) with age. However, simple

comparison is impossible, given that the study in reference reported increasing frequency based solely on 4 hours of observation (Chun et al., 1988). In the present study, although aged rats exhibited a large variance in voiding frequency, only two of twelve aged rats showed elevated frequency of urination, while the majority of aged rats exhibited decreased voiding frequency compared to young rats. Taking these matters into account, it can be presumed that the voiding frequency was decreased, not increased, by aging.

4.2 KCl-induced Contraction in Young and Aged Rat Bladders

Urinary bladder function is altered in aged animals, including humans. The maximal urine flow rate and contraction of detrusor smooth muscle are decreased, while the voiding frequency and the residual volume are increased with aging (Homma et al., 2006; Pfisterer et al., 2006). A previous study reported an increase in smooth muscle cell hypertrophy and fibrosis in the bladder of aged rats (Aita et al., 2012). Consequently, hypertrophy and fibrosis of muscle cells by aging could be the reason why bladders of aged rats were heavier in our study. KCl-induced isotonic contraction previously has been shown to be unaltered by aging (Ordway et al. 1986; Saito et al., 1991; Munro and Wendt, 1993; Pagala et al., 2001; Yoshida et al., 2001; Oshiro et al., 2014), in contrast to the results of the present study. However, these earlier studies did not factor in the weight of each bladder strip. We found a significant difference between young and aged rats in terms of KCl-induced contractions when the data were normalized by strip length and weight.

4.3 Effect of 5-HT on Detrusor Smooth Muscle Contraction of Young and Aged Rat Bladder

The present work showed that the maximal contractile response to 5-HT did not differ significantly between aged and young rats, although the minimum concentration of 5-HT that induces contraction was greater, but not significantly, in aged rats. Additionally, tetrodotoxin inhibited 5-HT-induced contraction in the bladders of aged rats, but not in those of young rats. The 5-HT-induced contraction was not altered by denudation of the urothelium in the bladder of young rats (data not shown).

These findings suggested that the expression site and characteristics of 5-HT receptors can be affected in aged rats. In contrast to the present results, a previous study reported that the maximal contractile response to 5-HT, but not the minimum concentration which induces contraction by 5-HT, was greater in the aged rat bladder than in the young rat bladder (Pagala et al., 2001). That study employed female Wistar rats at 6, 16, and 24 months of age. In contrast, the present study used male Fischer 344 rats that were 2-3 and 26-30 months old. These differences in experimental conditions may be sufficient to explain the different results in aged rats on serotonergic contraction. In fact, different rat strains are known to exhibit distinct responses to noradrenaline. Specifically, bladders from young Fischer 344 rats have been shown to be contracted with high sensitivity by noradrenaline, while bladders from Wistar rats show a weak response to noradrenaline (Saito et al., 1993).

Contraction induced by 5-HT is also augmented in rats with BOO (Sakai et al., 2013b). In contrast, the present study reported that aged Fischer strain 344 rats needed higher concentration of 5-HT to contraction and did not exhibit an increasing voiding frequency. Consequently, in our aged rats, the bladder detrusor muscle may have been underactive, rather than overactive. Therefore, it can be presumed that 5-HT is involved in voiding frequency. Future studies will need to examine how 5-HT relates to voiding function.

4.4 Effects of 5-HT₂ Receptor Antagonists on 5-HT-induced Smooth Muscle Contraction in Young and Aged Rat Bladders

In our study, ketanserin, a 5-HT_{2A/2C} receptor antagonist, inhibited detrusor contraction in young and aged rats. The inhibition was observed with concentrations at 1 and 3 μ M ketanserin. Naftopidil did not change the entire CRC of its vehicle significantly. Originally, naftopidil was identified as an adrenergic α -1D and α -1A subtype-selective antagonist (Mittra et al., 2007), but this compound also has 5-HT_{2A/2B} antagonistic activity. A previous report by Sakai et al. showed that naftopidil produced a rightward shift of the concentration-response curve to 5-HT in young female Sprague-Dawley rats (Sakai et al., 2013a), an observation that contrasts with our results. Those authors reported that naftopidil binds to the human 5-HT_{2A} and 5-HT_{2B} receptors, which are expressed in HEK-293 and CHO cells, with pK_i values of 6.55 and 7.82, respectively, and that this compound inhibits 5-HT₂ receptor agonist-induced bladder contractions. Those

authors also found that 5-HT-induced rat bladder contraction was inhibited by ketanserin. The inability of naftopidil to inhibit bladder contraction in the present study may reflect changes in 5-HT_{2B} receptor subtypes in aged Fischer 344 rats.

Another reason for the discrepancy in the effect of 5-HT₂ receptor antagonists may be the use of tetrodotoxin treatment in our experiment. In the present study, we used tetrodotoxin when the concentration-response curve of 5-HT was determined to exclude the possible involvement of neurotransmitters released from nerve endings by 5-HT stimulation.

4.5 Role of 5-HT in Atropine-resistant Contractions in the Aged Rat Bladder

In the human urinary bladder, EFS-induced neurogenic contractions contain several components, including both cholinergic and purinergic contractions (Yoshida et al., 2001). Aging does not alter frequency-dependent contraction by EFS or smooth muscle contraction by either a muscarinic agonist (carbachol) or a purinergic agonist (ATP). In the same study, it also was reported that atropine-resistant contraction of the human bladder is greater in people older than 70 years (Yoshida et al., 2001). Additionally, the purinergic component of EFS-induced contraction is greater in aged than in young people, and is responsible for atropine-resistant contraction in aged humans. In the present study, we examined the possible involvement of the serotonergic component in atropine-resistant contraction. However, the 5-HT₂ receptor antagonist naftopidil did not inhibit EFS-induced contraction, suggesting the absence of 5-HT₂-induced contraction in atropine-resistant contraction. The effect of ketanserin on EFS-induced contraction is difficult to explain. EFS-induced contraction was not inhibited, but instead augmented by ketanserin, while the ketanserin vehicle (DMSO) also potentiated EFS-induced contraction in young rats (data not shown). This finding suggested that the potentiating effect of ketanserin may be due, at least in part, to DMSO. Therefore, the induction of atropine-resistant contraction by EFS in rat detrusor muscle was not due to an increased component of 5-HT-sensitive contraction. On the other hand, a recent study suggested that ketanserin and naftopidil enhance detrusor contractility (Hattori et al., 2017). The precise reason for the potentiating effect of ketanserin on EFS-induced contraction remains unclear and will require further study.

4.6. Limitations

Several limitations are included in the present study. First, the present in vitro work was conducted to pharmacologically determine the effect of 5-HT receptors in the aged detrusor; however, in vivo studies are needed, especially those using cystometric procedures. Second, we determined the difference between ketanserin and naftopidil on detrusor contraction induced by 5-HT. The difference is possibly caused by selectivity of each drug for specific 5-HT receptor subtypes. Further studies are needed to clarify the details of these differences in drug selectivity. Thirdly, this study lacked a mature group (10-15 months old) and a much older group (≥ 30 months old), thereby segregating aging from senescence, to cover changes that occur during aging.

5. CONCLUSION

In this study, we investigated whether voiding frequency and 5-HT mediated contraction in the detrusor smooth muscle of the bladder is altered in aged rats; and whether 5-HT is involved in neurogenic contraction, especially in atropine-resistant contraction. Our results showed that volume per micturition increases, while urination frequency decreases in aged rats. In addition, urination intervals during active periods are extended in aged rats, indicating that the urination rhythm changes between light and dark cycles in aged rats. Moreover, the minimum concentrations of 5-HT needed to induce contraction was higher in aged rats than in young rats, but the maximal contraction in response to 5-HT is unchanged. Naftopidil-sensitive contraction is diminished in aged detrusor muscle. The results of EFS-induced neurogenic contraction experiments suggested that atropine-resistant contraction is not due to an increased component of 5-HT-sensitive contraction. In future studies, we propose to measure differences in other subtypes of receptors between young and aged rats, and to investigate the role of 5-HT in voiding function.

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Conflict of Interest.

Tsuyoshi Hattori is an employee of Asahi Kasei Pharma Corporation. Asaki Takanashi, Azuki Sakai-Saito, Sumika Kanno-Saito, Yumi Katano, and Takao Okada have no conflicts of interest or financial relationships to declare.

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