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Emotional Stress Facilitates Micturition Reflex: Possible Inhibition by an α_1-Adrenoceptor Blocker in the Conscious and Anesthetized State

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Purpose: To test the hypothesis that naftopidil prolongs intercontraction intervals in rats undergoing chronic stress as observed in previous animal models, voiding behavior and bladder function were measured and analyzed.
Methods: Female Sprague-Dawley rats weighing 200–230 g were exposed to repeated variate stress (RVS) for 1 week, chronic variable mild stress for 2 weeks, or simple mild stress for 1 week. Voiding behavior was assessed in metabolic cages. Voiding frequency and urine output were measured, and changes of these values were compared for the different types of stress. Micturition reflex was analyzed using unconscious cystometry. Naftopidil was administered orally at 30 mg/kg/day for 2 weeks.
Results: Unexpectedly, no stress-exposed rats exhibited increased micturition frequency compared to the normal nonstressed control. However, intercontraction intervals were shortened with each type of stress in the unconscious condition, especially by RVS ($P < 0.01$). Naftopidil prolonged the shortened intervals.
Conclusions: Although voiding behavior appears approximately normal in rats chronically exposed to emotional stress, internal bladder function can be affected. With anesthesia, micturition intervals were moderately shortened by emotional stress and clearly improved by naftopidil. Therefore, naftopidil appears to act at the spinal level at least.

Keywords: Chronic stress; Lower urinary tract symptoms; Naftopidil; Urinary bladder; Voiding

- **Research Ethics:** The study protocol (No. 5804) was approved by the President of the University of the Ryukyus based on the judgment of the institutional Animal Care and Use Committee.
- **Conflict of Interest:** TH, the first author and corresponding author, belongs to Asahi Kasei Pharma Corporation. This study was supported by Asahi Kasei Pharma Corporation.

- ***HIGHLIGHTS***
  - Chronic emotional stress can alter urinary bladder function at the peripheral level by shortening intercontraction intervals.
  - The central nervous system may suppress stress-associated bladder dysfunction.
  - Naftopidil prolongs the intercontraction intervals shortened by stress.

INTRODUCTION

Micturition and emotion are closely linked [1]. Micturition is coordinated not in the spinal cord but in the brainstem, where it is closely associated with the limbic system [1]. As previous research indicated that psychotherapy improves nocturia and

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incontinence in patients with sensory urgency and detrusor instability [2], the association between mental state and lower urinary tract symptoms (LUTS) has been studied. Research indicates that the periaqueductal grey (PAG), pontine micturition center (PMC), and forebrain act in concert to modulate micturition during psychological stress [3-5]. The PMC, particularly Barrington's nucleus, was identified as playing a pivotal role in coordinating forebrain and colonic/bladder pelvic viscera activity by bidirectional projections extending to the locus coeruleus and spinal cord [6,7].

The mechanisms of stress-associated voiding abnormalities have been investigated using experimental animal models. In male rhesus macaques subjected to social stress associated with the intruder paradigm, anxiety and fear-related behaviors such as urination were observed, and corticotropin-releasing hormone was found to play a crucial role in the physiologic response to psychological stress [8]. Neonatal maternal separation (NMS) induces an increase in voiding frequency and degranulation of mast cells in the urinary bladder, resulting in increased urinary bladder sensitivity compared to naive animals. However, compared to rats not exposed to exercise, voluntary wheel running attenuates voiding dysfunction and the activation of mast cell in rats subjected to NMS [9]. Water avoidance stress increases the micturition frequency in female rats but decreases it in male mice [10,11]. In female rats, the inflammation score and the number of degranulated mast cells in the bladder wall are higher compared with sham-treated rats [11]. Repeated variate stress (RVS) decreases the intercontraction interval, voided volume, and bladder capacity, although micturition pressure and baseline pressure are unchanged. RVS also increases the amounts of myeloperoxidase, nerve growth factor, and histamine in the urinary bladder [12]. Chronic variable mild stress (CVMS) increases the level of serum corticosterone and decreases the body weight in both males and females [13]. In the hypothalamic paraventricular nucleus, CVMS increases levels of corticotropin-releasing factor (CRF) mRNA in males and decreases CRF peptide in females; this factor coordinates brain noradrenergic activity and the sacral parasympathetic system [6].

The presence of LUTS in elderly men can be suggestive of benign prostatic hyperplasia, which presents with urgency, nocturia and hesitancy as the most bothersome symptoms [14]. To manage LUTS, α1-adrenoceptor antagonists are ordinarily prescribed, and these agents improve both voiding and storage symptoms. Animal experiments suggested that the mechanism of α1-adrenoceptor antagonists involves a decrement in urethral pressure and suppression of the micturition reflex [15,16]. Administration of the α1-adrenoceptor antagonists naftopidil intrathecally at the L6-S1 level suppresses the micturition reflex via modulation of glycine and GABA receptors [17]. Naftopidil improves shortening of intercontraction intervals in rat models of pelvic venous congestion, interstitial cystitis, bladder outlet obstruction, and cold stress [18-21]. Based on these data, we hypothesized that naftopidil also prolongs intercontraction intervals in rats subjected to chronic stress. To test this hypothesis, we exposed rats to three types of stress and assessed voiding behavior and urinary bladder function.

MATERIALS AND METHODS

Animals

A total of 77 female Sprague-Dawley rats weighing 200–230 g were used in the study. Rats were housed at a constant temperature (23°C ± 2°C) and relative humidity (55% ± 15%) under a regular light-dark schedule (light period 08:00 AM to 20:00 PM) with food and water freely available except during the time of the study tests.

Study Design

The design of the current study is summarized in Fig. 1. The study consisted of 3 sets of experiments involving different types of chronic stress. In each experiment, rats were divided into 4 groups (n = 6–8 in each group): group 1, control without stress or naftopidil (α1-adrenoceptor blocker); group 2, administration of naftopidil without stress; group 3, subjected to stress but not treated with naftopidil; group 4, subjected to stress and treated with naftopidil. After the stress period, voiding behavior was assessed, and the micturition reflex was analyzed. The effect of naftopidil on various parameters related to urinary bladder function was also assessed.

Chronic Stress Models

Table 1 summarizes the three different types of chronic stress applied in the study. First, rats were subjected to CVMS for 2 weeks, as described previously [13,22]. Rats were sequentially subjected to each of the following stressors: forced to swim at 4°C for 2 minutes or at 12°C for 4 minutes; exposed to humid sawdust for 3 hours, overnight, or 24 hours; fasted and deprived of water overnight or for 24 hours; conditioned to the light and dark cycle, and then isolated overnight or for 15 minutes at 4°C.

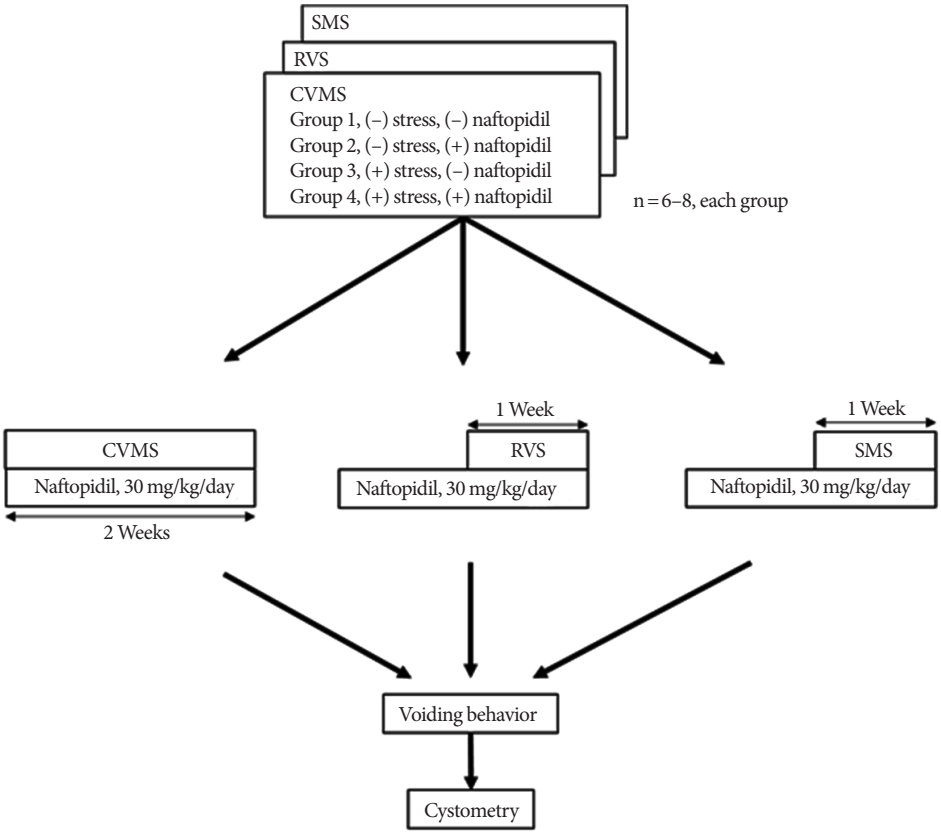

Fig. 1. Schematic illustration of the design of the current study. Rats were divided into 3 groups: chronic variable mild stress (CVMS), repeated variate stress (RVS), and simple mild stress (SMS). In each stress group, the rats were further divided into 4 subgroups: groups 1 through 4. After the stress period, voiding behavior was assessed and analyzed, followed by cystometry.

Second, rats were exposed to RVS for 1 week, as described previously, with minor modifications [12]. Rats were sequentially subjected to each of the following stressors: oscillation at 60–120 rpm for 30 minutes in a plastic chamber secured on a rotator (SRR-2; AS ONE Corp., Osaka, Japan); forced swimming for 5 minutes in a cylinder filled with room-temperature water to a depth that prevented the tail from touching the bottom of the container; electrical foot-shocks (which make the rats jump promptly) twice for 5 seconds at 100 V with a 1-minute interval between shocks in a dedicated chamber box (custom built); restraint in a cylindrical device (9×15 cm [D×H]) (homemade) for 60 minutes. Third, rats were exposed to simple mild stress (SMS) consisting of lighting or humid sawdust for 24 hours every other day for 1 week. Rats were sequentially subjected to the following stressors: lighting overnight; humid sawdust for 24 hours every other day. After each stress period, voiding behavior and urinary bladder function were assessed.

Voiding Behavior

Rats were placed in metabolic cages (CL-0353; CLEA Japan Inc., Tokyo, Japan) with free access to standard food and water for 24 hours. The cumulative weight of urine produced by each rat was measured by the electronic balance (AD-1688; A&D Company, Ltd., Tokyo, Japan) and recorded on the connected computer (PC-LS150F2P2W; NEC Corp., Tokyo, Japan) each minute during the assessment. Voided urine volume and times were individually determined during the 12-hour dark period from 20:00 PM to 08:00 AM, the 12-hour light period from 08:00 AM to 20:00 PM, and the total 24-hour period.

Continuous Cystometry

Rats were anesthetized with urethane (0.4 g/kg intraperitoneally and 0.8 g/kg subcutaneously) and placed in restraining cages (NAIGAI-CFK-1P, NMS, Tokyo, Japan). A polyethylene catheter (PE-50; Clay Adams, Parsippany, NJ, USA) connected to an infusion pump was inserted transurethrally into the bladder of

Table 1. Outline of stress exposure

Day	Stressor	Duration
Chronic variable mild stress		
1	Swim (4°C), humid sawdust	2 min, 3 hr
2	Fasting and water deprivation	24 hr
3	Lights on, humid sawdust	Overnight, 24 hr
4	Lights off, swim (4°C)	3 hr, 2 min
5	Fasting and water deprivation, isolation	Both overnight
6	Cold isolation (4°C), lights off	15 min, 2 hr
7	Swim (12°C), fasting and water deprivation	4 min, overnight
8	Inverted light/dark cycle, humid sawdust	-, overnight
9	Lights on, fasting and water deprivation	Both overnight
10	Lights off, humid sawdust	3 hr, 24 hr
11	Isolation, fasting and water deprivation	Both overnight
12	Restraint, light on	1 hr, overnight
13	Inverted light/dark cycle, fasting and water deprivation	-, overnight
14	Humid sawdust, restraint	3 hr, 1 hr
Repeated variate stress		
1	Oscillation	30 min
2	Swim	5 min
3	Foot shock	5 sec (twice)
4	Restraint	1 hr
5	Swim	5 min
6	Foot shock	5 sec (twice)
7	Restraint	1 hr
Simple mild stress		
1	Lights on	Overnight
2	Humid sawdust	Overnight
3	Lights on	Overnight
4	Humid sawdust	Overnight
5	Lights on	Overnight
6	Humid sawdust	Overnight
7	Lights on	Overnight

each animal. Physiologic saline was infused into the urinary bladder (0.05 mL/min), and bladder activity was monitored via the urethral catheter, which was connected to a pressure transducer and saline infusion pump via a 3-way stopcock. The bladder contraction interval, intravesical baseline pressure, and maximum bladder pressure were measured during the final 30–45 minutes of cystometry, which lasted at least 90 minutes.

Drug Administration

Naftopidil (Asahi Kasei Pharma Corp., Tokyo, Japan) was administered orally at 30 mg/kg/day via the food at 0.04% (w/w) for 2 weeks. In the case of CVMS, naftopidil administration was started at the beginning of the stress period. For week-long RVS or SMS experiments, naftopidil administration was started 1 week before the start date of the stress period.

Statistical Analysis

Results are shown as the mean ± standard error of the mean. We primarily compared groups 1 and 3 and groups 3 and 4. In terms of multiplicity, data were analyzed using the Tukey-Kramer test. $P < 0.05$ was considered indicative of statistical significance. All analyses were performed using JMP ver. 14 (SAS Institute, Cary, NC, USA).

RESULTS

Voiding Behavior

Data regarding voiding behavior after exposure to each type of stress are summarized in Table 2. In the CVMS model, the stress did not change the frequency of micturition between group 1 and 3 during the dark, light, or 24-hour periods. In the RVS model, the frequency of micturition of group 3 was signifi-

Table 2. Voiding behavior after exposure to each stress during dark, light, and 24-hour periods

Variable	Group 1	Group 2	Group 3	Group 4
Chronic variable mild stress				
Frequency of micturition				
Dark	27.8 ± 2.6 (21.5–34.9)	21.3 ± 2.0 (16.5–26.1)	34.0 ± 3.2 (26.4–41.6)	30.5 ± 2.6 (24.2–36.8)
Light	12.6 ± 1.9 (8.2–17.1)	10.0 ± 0.4 (9.1–10.9)	14.9 ± 1.7 (10.9–18.8)	12.1 ± 1.4 (8.7–15.5)
24 hr	39.6 ± 4.0 (30.1–49.1)	31.3 ± 2.3 (25.8–36.8)	45.9 ± 4.2 (35.6–56.2)	42.6 ± 3.9 (33.4–51.8)
Urine volume (mL)				
Dark	27.7 ± 2.0 (22.9–32.4)	21.5 ± 2.2 (16.1–26.8)	30.1 ± 8.3 (23.2–37.0)	28.2 ± 2.8 (21.7–34.8)
Light	12.8 ± 1.3 (9.7–15.9)	10.2 ± 0.8 (8.3–12.1)	13.3 ± 1.2 (10.4–16.2)	13.4 ± 1.3 (10.3–16.5)
24 hr	40.5 ± 3.2 (33.0–48.0)	31.7 ± 2.6 (25.3–38.0)	43.3 ± 4.1 (33.7–53.0)	38.9 ± 3.3 (30.9–46.8)
Repeated variate stress				
Frequency of micturition				
Dark	20.7 ± 2.0 (15.8–25.6)	14.1 ± 1.7 (10.1–18.2)	8.7 ± 1.1** (6.0–11.4)	8.0 ± 1.5 (4.6–11.4)
Light	7.6 ± 1.1 (4.8–10.3)	9.0 ± 0.5 (7.7–10.3)	7.4 ± 0.7 (5.7–9.2)	6.3 ± 0.6 (4.9–7.6)
24 hr	28.3 ± 2.3 (22.5–34.0)	23.1 ± 2.0 (18.4–27.9)	16.1 ± 1.5** (12.4–19.9)	14.3 ± 1.8 (9.9–18.6)
Urine volume (mL)				
Dark	11.0 ± 1.3 (7.9–14.1)	8.3 ± 1.8 (4.0–12.5)	4.0 ± 0.4** (3.0–4.9)	3.0 ± 0.5 (1.8–4.1)
Light	6.9 ± 0.9 (4.6–9.2)	8.2 ± 0.8 (6.3–10.0)	4.6 ± 0.4 (3.6–5.6)	3.7 ± 0.4 (2.6–4.7)
24 hr	17.9 ± 1.3 (14.7–21.1)	16.4 ± 2.3 (10.9–21.9)	8.6 ± 0.7** (6.9–10.3)	6.6 ± 0.8 (4.7–8.6)
Simple mild stress				
Frequency of micturition				
Dark	20.7 ± 2.0 (15.8–25.6)	14.1 ± 1.7 (10.1–18.2)	13.9 ± 2.1 (8.9–18.9)	13.5 ± 2.3 (7.9–19.0)
Light	7.6 ± 1.1 (4.8–10.3)	9.0 ± 0.5 (7.7–10.3)	9.8 ± 1.4 (6.4–13.1)	10.8 ± 1.1 (8.0–13.5)
24 hr	28.3 ± 2.3 (22.5–34.0)	23.1 ± 2.0 (18.4–27.9)	23.6 ± 3.4 (15.6–31.7)	26.1 ± 2.7 (19.8–32.4)
Urine volume (mL)				
Dark	11.0 ± 1.3 (7.9–14.0)	8.3 ± 1.8 (4.0–12.5)	10.0 ± 1.3 (7.0–13.1)	8.9 ± 1.2 (6.0–11.8)
Light	6.9 ± 0.9 (4.6–9.2)	8.2 ± 0.8 (6.3–10.0)	9.9 ± 2.2 (4.8–15.0)	10.2 ± 0.8 (8.2–12.2)
24 hr	17.9 ± 1.3 (14.7–21.1)	16.4 ± 2.3 (10.9–21.9)	19.9 ± 3.2 (12.3–27.6)	19.1 ± 1.6 (15.4–22.8)

Values are presented as mean ± standard error of the mean (95% confidence interval) (n = 7–8).
Group 1, control without stress or naftopidil; group 2, administration of naftopidil without stress; group 3, stress exposure but no naftopidil; group 4, both stress exposure and naftopidil.
**P < 0.01 vs. group 1 by Tukey-Kramer test.

Table 3. Cystometric analyses after stress exposure

Variable	Group 1	Group 2	Group 3	Group 4
Chronic variable mild stress				
Intercontraction interval (min)	20.8 ± 1.4 (17.5–24.2)	23.3 ± 2.7 (16.6–29.9)	15.4 ± 2.0 (10.2–20.5)	22.2 ± 1.4 (18.6–25.8)
Baseline pressure (cm H_2O)	4.6 ± 0.3 (3.9–5.4)	4.3 ± 0.3 (3.6–5.0)	4.7 ± 0.6 (3.2–6.3)	4.7 ± 0.4 (3.6–5.9)
Maximum pressure (cm H_2O)	45.5 ± 3.2 (37.7–53.4)	49.1 ± 5.5 (35.6–62.6)	50.4 ± 4.0 (40.0–60.7)	54.1 ± 2.2 (48.5–59.8)
Repeated variate stress				
Intercontraction interval (min)	18.8 ± 1.2 (16.0–21.6)	20.1 ± 1.2 (17.1–23.2)	13.2 ± 0.9** (11.1–15.2)	19.3 ± 0.9‡ (17.2–21.5)
Baseline pressure (cm H_2O)	4.9 ± 0.4 (4.0–5.8)	5.4 ± 0.5 (4.1–6.7)	5.7 ± 0.4 (4.9–6.6)	5.3 ± 0.5 (4.0–6.6)
Maximum pressure (cm H_2O)	45.4 ± 2.1 (40.6–50.3)	47.8 ± 3.6 (39.4–56.2)	36.3 ± 2.0 (31.5–41.0)	36.4 ± 1.6 (32.5–40.3)
Environmental stress				
Intercontraction interval (min)	18.8 ± 1.2 (16.0–21,6)	20.1 ± 1.2 (17.1–23.2)	15.4 ± 1.5 (12.0–18.9)	22.3 ± 1.1† (19.6–24.9)
Baseline pressure (cm H_2O)	4.9 ± 0.4 (4.0–5.8)	5.4 ± 0.5 (4.1–6.7)	6.4 ± 0.4 (5.4–7.3)	6.0 ± 0.6 (4.5–7.5)
Maximum pressure (cm H_2O)	45.4 ± 2.1 (40.6–50.3)	47.8 ± 3.6 (39.4–56.2)	36.8 ± 2.2 (31.6–42.1)	40.5 ± 1.9 (35.8–45.1)

Values are presented as mean ± standard error of the mean (95% confidence interval) (n = 6–8).
Group 1, control without stress or naftopidil; group 2, administration of naftopidil without stress; group 3, stress exposure but no naftopidil; group 4, both stress exposure and naftopidil.
**P < 0.01 vs. group 1, and †P < 0.05, ‡P < 0.01 vs. group 3 by Tukey-Kramer test.

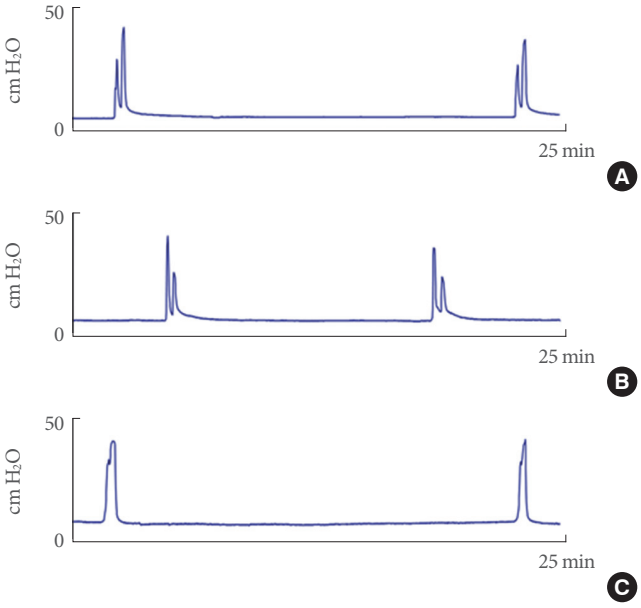

Fig. 2. Typical cystometrograms from repeated variate stress (RVS) experiments. Upper, middle, and lower panels show the bladder pressure in group 1 (control, A), group 3 (stress exposed, B), and group 4 (stress exposed with naftopidil administration, C), respectively.

cantly lower than that of group 1 during the dark and 24-hour periods ($P<0.01$ each). Also, the voided volume in group 3 was significantly lower than that of group 1 during the dark and 24-hour periods ($P<0.01$ each). In the SMS model, the stress did not affect the frequency of micturition in group 1 or 3 during the dark, light, or 24-hour periods.

Continuous Cystometry

Data regarding unconscious cystometry after exposure to each type of stress are summarized in Table 3, and typical cystometrograms are shown in Fig. 2. In each of the stress models, the intercontraction interval tended to decrease in group 3 compared to group 1, and the difference between group 1 and 3 in the RVS model was statistically significant ($P<0.01$). Naftopidil significantly prolonged the decreased intervals in the RVS and SMS models ($P<0.01$ and 0.05, respectively) but not in the CVMS model. Baseline and maximum bladder pressure were not affected by any of the stress types examined.

DISCUSSION

In the present study, three types of stress were examined. Analysis of voiding behavior indicated that these stresses unexpectedly did not increase the micturition frequency, and RVS significantly decreased the micturition frequency during daytime and over the 24-hour period. However, cystometry experiments showed that the stresses examined, particularly RVS, significantly shortened the inter-contraction intervals. Naftopidil prolonged the decreased intervals.

Experimental Stress Model and Urinary Bladder Function

Previous research demonstrated that chronic stress modulates bladder function; water avoidance stress increases voiding frequency and shortens the voiding interval [23]. Social stress leads to urinary retention, decreased voiding frequency, and increases in voiding interval and urine volume [24,25]. RVS shortens the voiding interval and decreases the voided volume [12]. These data suggest that different types of stress lead to different dysfunctions in the urinary bladder. As a high micturition frequency generally indicates shortening of the intercontraction interval, there is a discrepancy in our present results between no change in voiding frequency (Table 2) and shortened contraction intervals (Table 3). The rather complex reasons for this discrepancy can be explained as follows. First, the central nervous system can strongly suppress stimulation of the micturition reflex as a mechanism for maintaining continence as the urinary bladder is filling, via neuronal projections connecting the PAG, PMC and forebrain [1]. Limbic structures prevent the PAG from exciting the PMC neurons, even when other parts of the PAG receive strong signals from the sacral cord that the urinary bladder is full and need to be emptied as soon as possible [26]. Medial frontal lobe neurons excited by glutamate inhibit the micturition reflex [27]. If this system operates under stressful condition such as those employed in the present study, and even if the emotional stress practically induces the micturition reflex, as indicated by Smith et al. [23], significant inhibition of the PAG and/or PMC may occur. Total voiding behavior is assumed to be balanced around normal micturition. This could explain why it appeared that micturition was not facilitated in group 3 as compared to group 1. We also speculate that together with enhancement of the micturition reflex brought on by emotional stress, activity of the central nervous system and/or limbic system can be increased to suppress the micturition reflex to maintain biophylaxis when in a conscious or awakened state. In our cystometry experiments, the animals were anesthetized; hence, the activity of the central nervous system and/or limbic system could have been sup-

pressed, such that only the emotional stress had an effect on micturition frequency and voiding behavior. Regarding the condition of cystometry with the isovolumetric procedure under urethane anesthesia, MK-801 inhibits the reflex of bladder contractions, showing that glutamatergic excitatory mechanisms are essential for micturition [28]. MK-801 also facilitates micturition for initiation of the contraction reflex in unanesthetized decerebrate rats, indicating glutamate-mediated inhibitory control. These results at least partly support the above rationale. Secondly, the emotional stress could have been too weak and thus, inadequate for rats in experiments in a conscious state, as shortening of inter-contraction intervals was observed in the anesthetized animals. However, at least in the RVS model, as total urine output decreased in animals housed in metabolic cages (Table 2), we suspect that the emotional stress could have caused a depression-like state and consequent decrease in water intake. Therefore, speculation regarding weakness of the stressors can be excluded.

Experimental Stress Model and Changes in Bladder Tissue

In the present study of the effect of chronic stress on urinary bladder function, the micturition reflex was facilitated, even in anesthetized animals. This observation, supported by the results of previous studies, suggests that a functional change was generated in peripheral tissues, the urinary bladder, and/or the primary afferent nerve. In the urinary bladder, the number of mast cells and the inflammation score are increased by water avoidance stress [11]; histamine and myosin heavy chain are overexpressed; BrdU incorporation is increased by social stress [29]; and myeloperoxidase and nerve growth factor are upregulated by RVS [12]. In the hypothalamic paraventricular nucleus, CVMS increases expression of CRF mRNA in males and decreases expression of CRF peptide in females [13]. CRF is prominently expressed in the descending pathway from Barrington's nucleus to the sacral parasympathetic nucleus (SPN) in the lumbosacral spinal cord, and prominent CRF immunoreactivity can be seen in the SPN of adult rats [30]. Therefore, CVMS may suppress the inhibition of micturition frequency by CRF at the lumbosacral spinal level.

Social stress was shown to increase both the intercontraction interval and bladder capacity. The mechanism involves upregulation of CRF mRNA for expression in Barrington's nucleus, which projects to the pelvic organs such as the urinary bladder and colon, where CRF suppresses the activity of afferent nerves, resulting in enlargement of bladder capacity and the increment of intercontraction intervals [25,31]. Social stress elevates intracellular calcium levels, which in turn leads to an increase in the level of calmodulin-calcium complexes. Then, calcineurin dephosphorylates a nuclear transcription factor of activated T cells. As a result, changes in myosin isoforms in the urinary bladder and bladder fibrosis develop [31]. In contrast to social stress, repeated restraint-associated stress does not affect urinary bladder function [25]. Although the stresses examined in the present study shortened intercontraction intervals, the mechanism remains unclear. As unpredictable stress can alter gamma-aminobutyric acid-ergic (GABAergic) receptor expression [32], a change in GABAergic transmission may shorten the intervals in response to stress, particularly RVS.

Effect of Naftopidil on Bladder Function

During bladder filling, higher brain centers such as the prefrontal cortex suppress excitatory signals to the PMC to prevent abnormal voiding and incontinence [1]. Nishijima et al. [27] found that medial frontal lobe neurons excited by glutamate inhibit the micturition reflex via activation of the rostral pontine reticular formation via glutamatergic projections in conscious cystometry, whereas medial frontal lobe neurons excited by noradrenaline stimulate the micturition reflex. Micturition intervals are also prolonged by naftopidil administered in the medial frontal lobe instead of noradrenaline. Intrathecal administration of α1-adrenoceptor antagonists such as tamsulosin, silodosin, and BMY7378 prolongs micturition intervals [33]. These results suggest that naftopidil suppresses the micturition reflex at a higher brain center and/or spinal cord level.

The PMC projects directly into the parasympathetic bladder motor neurons and sacral GABAergic and glycinergic premotor interneurons that inhibit motor neurons in Onuf's nucleus [1]. In anesthetized animals, intrathecal injection of naftopidil suppresses the micturition reflex in isovolumetric cystometry [16]. Abolishment of urinary bladder contractions by intrathecal administration of naftopidil is antagonized by simultaneous intrathecal administration of strychnine and/or bicuculine [17]. These results are supported by data from *in vitro* patch-clamp experiments in which naftopidil was shown to enhance inhibitory post-synaptic current in slices of lumbosacral spinal cord in rats [34]. These effects of naftopidil could be antagonized by strychnine or bicuculine as well. Therefore, bladder contraction caused by PMC stimulation may be suppressed by strengthening glycinergic and/or GABAergic input at the spinal level.

In conclusion, although voiding behavior may appear nor-

mal during chronic exposure to emotional stress, internal bladder function can be affected. Because the current results in terms of voiding behavior, which was assessed in the conscious state, were not ideal for evaluating the effect of emotional stress, we could not conclusively elucidate the mechanism of action of naftopidil in the brain center. In contrast, with anesthesia, micturition intervals were moderately shortened by emotional stress and definitely improved by naftopidil. This suggests that naftopidil acts at least at the spinal level. To treat LUTS associated with chronic emotional stress, enhancement of GABAergic or glycinergic input at the spinal level using naftopidil, for example, could be a viable treatment.

Limitations

Although several physiologic and behavioral parameters were assessed in the present study, experiments examining the effects of stress at the molecular level were not conducted. Future studies should thus evaluate the effects of chronic stress on urinary bladder function at the molecular level. Although urine volume was measured and discussed, the volume of water intake was not assessed in the present study.

AUTHOR CONTRIBUTION STATEMENT

- Full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis: *KS*
- Study concept and design: *KS*
- Acquisition of data: *SN*
- Analysis and interpretation of data: *SN, KS*
- Drafting of the manuscript: *TH*
- Critical revision of the manuscript for important intellectual content: *KS*
- Statistical analysis: *TH*
- Obtained funding: *KS*
- Administrative, technical, or material support: *TU, KK*
- Study supervision: *HY*

REFERENCES

1. Holstege G. Micturition and the soul. J Comp Neurol 2005;493:15-20.
2. Macaulay AJ, Stern RS, Holmes DM, Stanton SL. Micturition and the mind: psychological factors in the aetiology and treatment of urinary symptoms in women. Br Med J (Clin Res Ed) 1987;294:540-3.
3. Holstege G. The emotional motor system in relation to the supraspinal control of micturition and mating behavior. Behav Brain Res 1998;92:103-9.
4. Corigliano T, Renella R, Robbiani A, Riavis M, Bianchetti MG. Isolated extraordinary daytime urinary frequency of childhood: a case series of 26 children in Switzerland. Acta Paediatr 2007;96:1347-9.
5. Lee KS, Yoo TK, Liao L, Wang J, Chuang YC, Liu SP, et al. Association of lower urinary tract symptoms and OAB severity with quality of life and mental health in China, Taiwan and South Korea: results from a cross-sectional, population-based study. BMC Urol 2017;17:108.
6. Valentino RJ, Chen S, Zhu Y, Aston-Jones G. Evidence for divergent projections to the brain noradrenergic system and the spinal parasympathetic system from Barrington's nucleus. Brain Res 1996;732:1-15.
7. Pavcovich LA, Yang M, Miselis RR, Valentino RJ. Novel role for the pontine micturition center, Barrington's nucleus: evidence for coordination of colonic and forebrain activity. Brain Res 1998;784:355-61.
8. Habib KE, Weld KP, Rice KC, Pushkas J, Champoux M, Listwak S, et al. Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. Proc Natl Acad Sci U S A 2000;97:6079-84.
9. Pierce AN, Eller-Smith OC, Christianson JA. Voluntary wheel running attenuates urinary bladder hypersensitivity and dysfunction following neonatal maternal separation in female mice. Neurourol Urodyn 2018;37:1623-32.
10. McGonagle E, Smith A, Butler S, Sliwoski J, Valentino R, Canning D, et al. Water avoidance stress results in an altered voiding phenotype in male mice. Neurourol Urodyn 2012;31:1185-9.
11. Bazi T, Hajj-Hussein IA, Awwad J, Shams A, Hijaz M, Jurjus A. A modulating effect of epigallocatechin gallate (EGCG), a tea catechin, on the bladder of rats exposed to water avoidance stress. Neurourol Urodyn 2013;32:287-92.
12. Merrill L, Malley S, Vizzard MA. Repeated variate stress in male rats induces increased voiding frequency, somatic sensitivity, and urinary bladder nerve growth factor expression. Am J Physiol Regul Integr Comp Physiol 2013;305:R147-56.
13. Sterrenburg L, Gaszner B, Boerrigter J, Santbergen L, Bramini M, Elliott E, et al. Chronic stress induces sex-specific alterations in methylation and expression of corticotropin-releasing factor gene in the rat. PLoS One 2011;6:e28128.
14. Eckhardt MD, van Venrooij GE, van Melick HH, Boon TA. Prevalence and bothersomeness of lower urinary tract symptoms in be-

nign prostatic hyperplasia and their impact on well-being. J Urol 2001;166:563-8.
15. Takei R, Ikegaki I, Shibata K, Tsujimoto G, Asano T. Naftopidil, a novel alpha1-adrenoceptor antagonist, displays selective inhibition of canine prostatic pressure and high affinity binding to cloned human alpha1-adrenoceptors. Jpn J Pharmacol 1999;79:447-54.
16. Sugaya K, Nishijima S, Miyazato M, Ashitomi K, Hatano T, Ogawa Y. 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.
17. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Ueda T, Yamamoto H. Spinal mechanism of micturition reflex inhibition by naftopidil in rats. Life Sci 2014;116:106-11.
18. Chen Z, Ishizuka O, Imamura T, Aizawa N, Igawa Y, Nishizawa O, et al. Role of alpha1-adrenergic receptors in detrusor overactivity induced by cold stress in conscious rats. Neurourol Urodyn 2009; 28:251-6.
19. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Ueda T, Yamamoto H. Naftopidil improves locomotor activity and urinary frequency in rats with pelvic venous congestion. Biomed Res 2016;37: 221-6.
20. Majima T, Yamamoto T, Funahashi Y, Takai S, Matsukawa Y, Yoshida M, et al. Effect of naftopidil on bladder microcirculation in a rat model of bladder outlet obstruction. Low Urin Tract Symptoms 2017;9:111-6.
21. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Ueda T, Yamamoto H. Naftopidil improves symptoms in a rat model of tranilast-induced interstitial cystitis. Low Urin Tract Symptoms 2017;9:107-10.
22. Parihar VK, Hattiangady B, Kuruba R, Shuai B, Shetty AK. Predictable chronic mild stress improves mood, hippocampal neurogenesis and memory. Mol Psychiatry 2011;16:171-83.
23. Smith AL, Leung J, Kun S, Zhang R, Karagiannides I, Raz S, et al. The effects of acute and chronic psychological stress on bladder function in a rodent model. Urology 2011;78:967.e1-7.
24. Chang A, Butler S, Sliwoski J, Valentino R, Canning D, Zderic S. Social stress in mice induces voiding dysfunction and bladder wall remodeling. Am J Physiol Renal Physiol 2009;297:F1101-8.
25. Wood SK, Baez MA, Bhatnagar S, Valentino RJ. Social stress-induced bladder dysfunction: potential role of corticotropin-releasing factor. Am J Physiol Regul Integr Comp Physiol 2009;296:R1671-8.
26. Fowler CJ, Griffiths D, de Groat WC. The neural control of micturition. Nat Rev Neurosci 2008;9:453-66.
27. Nishijima S, Sugaya K, Kadekawa K, Ashitomi K, Yamamoto H. Effect of chemical stimulation of the medial frontal lobe on the micturition reflex in rats. J Urol 2012;187:1116-20.
28. Yoshiyama M, Roppolo JR, De Groat WC. Alteration by urethane of glutamatergic control of micturition. Eur J Pharmacol 1994;264: 417-25.
29. Mingin GC, Peterson A, Erickson CS, Nelson MT, Vizzard MA. Social stress induces changes in urinary bladder function, bladder NGF content, and generalized bladder inflammation in mice. Am J Physiol Regul Integr Comp Physiol 2014;307:R893-900.
30. Studeny S, Vizzard MA. Corticotropin-releasing factor (CRF) expression in postnatal and adult rat sacral parasympathetic nucleus (SPN). Cell Tissue Res 2005;322:339-52.
31. Long CJ, Butler S, Fesi J, Frank C, Canning DA, Zderic SA. Genetic or pharmacologic disruption of the calcineurin-nuclear factor of activated T-cells axis prevents social stress-induced voiding dysfunction in a murine model. J Pediatr Urol 2014;10:598-604.
32. Sarro EC, Sullivan RM, Barr G. Unpredictable neonatal stress enhances adult anxiety and alters amygdala gene expression related to serotonin and GABA. Neuroscience 2014;258:147-61.
33. Yoshizumi M, Matsumoto-Miyai K, Yonezawa A, Kawatani M. Role of supraspinal and spinal alpha1-adrenergic receptor subtypes in micturition reflex in conscious rats. Am J Physiol Renal Physiol 2010;299:F785-91.
34. Uta D, Xie DJ, Hattori T, Kasahara KI, Yoshimura M. Effects of naftopidil on inhibitory transmission in substantia gelatinosa neurons of the rat spinal dorsal horn in vitro. J Neurol Sci 2017;380:205-11.