Cold Stress Induces Lower Urinary Tract Symptoms (LUTS)

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Abstract

Cold stress due to whole body cooling at low environmental temperatures exacerbates lower urinary tract symptoms (LUTS) such as urinary urgency, nocturia, and residual urine. We established a model system using healthy conscious rats to explore the mechanisms of cold stress-induced detrusor overactivity. In this review, we summarize the basic findings revealed by this model.

Rats that are quickly transferred from room temperature (27±2ºC) to low temperature (4±2ºC) exhibit increased detrusor overactivity including increased basal pressure and decreased voiding interval, micturition volume, and bladder capacity. The cold stress-induced detrusor overactivity is mediated through a resiniferatoxin-sensitve C-fiber sensory nerve pathway involving α1-adrenergic receptors (ARs). Transient receptor potential melastatin 8 (TRPM8) channels, which are sensitive to thermal changes below 25-28ºC, also play an important role in mediating the cold stress responses. Additionally, the sympathetic nervous system is associated with transient hypertension and decreases of skin surface temperature that are closely correlated with the detrusor overactivity.

With this cold stress model, we show that α1-AR antagonists have the potential to treat cold stress-exacerbated LUTS. In addition, we show that traditional Japanese herbal mixtures composed of Hachimijiogan act, in part, by increasing skin temperature and reducing the number of cold sensitive TRPM8 channels in the skin. The effects of herbal mixtures have the potential to treat and/or prevent the exacerbation of LUTS by providing resistance to the cold stress responses. Our model provides new opportunities for utilizing animal disease models with altered lower urinary tract functions to explore the effects of novel therapeutic drugs. (248/250)

Key words: lower urinary tract symptoms, cold stress, detrusor overactivity, C-fiber, TRPM8 channel

Introduction

Cold stress produced by sudden change or continuous exposure to low environmental temperature seriously affects urinary tract functions along with other physiological responses.[1] For instance, whole body cooling increases heart rate and/or blood pressure.[2-4] Sudden whole body cooling elicits urinary sensations and frequent urination in healthy people, even in the absence of lower urinary tract symptoms (LUTS). For patients having LUTS, it especially exacerbates symptoms such as urinary urgency, nocturia, and residual urine. Thus, the response to cold stress potentially provides insightful understanding into the mechanism(s) of LUTS.[5-8] For this reason, we have established an animal model to investigate cold stress-exacerbated LUTS.[1]

Rats stimulated with cold stress show remarkable detrusor overactivity that causes an increase in basal pressure and decreases in voiding interval, micturition volume, and bladder capacity.[1] By using the cold stress model, we have found important aspects of the mechanism(s) that elicit cold stress-induced detrusor overactivity (Fig. 1). This review shows that cold stress-induced detrusor overactivity is mediated, at least in part, through a resiniferatoxin (RTX)-sensitive C-fiber sensory nerve pathway.[1] The response

is closely associated with the sympathetic nervous system that mediates transient hypertension[11] and sensory input from the lowered temperature of the skin. Other components of the response to cold stress include transient receptor potential melastatin 8 (TRPM8) channels within the skin cells and/or sensory neurons.[9,10] In addition, α1-adrenergic receptor (AR) antagonists have the potential to treat cold stress-exacerbated LUTS.[11,12] Finally, traditional Japanese herbal mixtures composed of Hachimijiogan have the potential to provide resistance to the cold stress responses.[13]

Cold stress-induced detrusor overactivity model

We produce environmental cold stress by quickly and smoothly transferring rats from room temperature (RT, 27±2ºC) to low temperature (LT, 4±2ºC) environments. Two days prior to cystometric investigations, the bladders are prepared by cannulation. The micturition patterns of conscious rats are monitored for 20 min prior to transfer to the LT environment. Immediately after transfer to the cold room, the rats exhibit violent shivering, pilomotor responses, and vigorous movements in the cages.

Changes in micturition patterns occur in two phases during the 40 min of exposure to LT (Fig. 2a). During the first 20 min after transfer, Phase I, the rats exhibit detrusor overactivity patterns such as increased micturition frequency (Fig. 2a, b), e.g., less than 5-min voiding interval, decreased micturition volume, and less than 1-ml bladder capacity (Fig. 2c). Basal pressure significantly increases, and micturition pressure tends to increase, though the change is not significant.[1] Also, residual volume does not change. During the second 20 min of LT exposure, Phase II, the detrusor overactivity patterns slowly mitigate and nearly disappear (Fig. 2a). The micturition patterns then approximate those at RT. The basal and micturition pressure and residual volume do not undergo significant further changes.[1] However, voiding interval (Fig. 2b), micturition volume, and bladder capacity (Fig. 2c) gradually increase, achieving levels that are significantly higher than in Phase I and similar, but somewhat lower, to those at RT. After 40 min of LT exposure, the rats are returned to RT. The detrusor overactivity patterns quickly and completely disappear (Fig. 2a), and all of the measured variables recover to the baseline RT values.

The transfers to and from the cold room are handled very gently and smoothly so as to avoid unnecessary stress in the rats. We have confirmed that these transfers do not affect their behavior. Throughout the cystometric investigations, we infuse an isotonic sodium chloride solution (saline) maintained at RT into the bladder at a rate of 10 ml per hour. We have confirmed that the temperature of the infused saline does not change during the LT exposure.[13] Additionally, we confirm that diuresis is not promoted under the LT condition.[11] Some studies from other groups directly stimulate the urinary bladder with an infusion of ice-cold water;[14-18] however, aside from our studies, there are no others that investigate the onset of urinary sensations and frequent urination induced by sudden whole body cooling. Our cold stress model accurately captures a common human experience, and the ensuing response of the rats is very similar to the human physiological response to similar conditions.

Relationship between cold stress-induced detrusor overactivity and C-fiber sensory nerve pathway

The activation of C-fiber sensory nerves is widely accepted as one of the mechanisms of LUTS exacerbation. We focused on RTX-sensitive C-fiber sensory nerve pathways to determine if they mediate the cold stress-induced detrusor overactivity. One day prior to the cystometric investigations, we subcutaneously inject 0.3 mg RTX/kg body weight into healthy rats.[1] That RTX dose decreases the S100- and CGRP-positive nerve fibers that are components of the C-fiber sensory nerves within the urinary bladder.[1] Cold stress cystometric investigations are then conducted as described above. While there are no significant differences between non-RTX-treated and RTX-treated rats at RT, the cold stress micturition patterns of the RTX-treated rats are different. The voiding interval (Fig. 2b), micturition volume, and bladder capacity (Fig. 2c) during LT Phase I of the RTX-treated rats are significantly greater than those of untreated rats. During LT Phase II, the micturition patterns and values of the RTX-treated rats are similar to those of LT Phase I. Further, due to the mitigation of responses that occurs in untreated rats during Phase II (Fig. 2c), there are no significant differences between any of the variables in the non-treated and the RTX-treated rats during the final 20 min of LT exposure. When the rats are returned to RT, the

micturition patterns of the RTX-treated rats recover to the RT patterns, which are similar to the untreated rats returned to RT. The RTX-dependent mitigation of detrusor overactivity induced during LT Phase I suggests that the C-fiber sensory nerve pathway mediates a portion of the cold stress-induced detrusor overactivity (Fig. 1).[1]

Cold stress-induced detrusor overactivity and blood pressure

A universally common experience is that whole body cooling due to exposure to low environmental temperature increases blood pressure[2,4] as well as urinary sensations and urinary frequency. By 10 min after transfer to LT, the blood pressure of rats significantly increases compared to that at RT, and it is maintained through the LT exposure period (Fig. 2d).[9] Thus, activity of sympathetic nervous system associated with transient hypertension partially correlates with the cold stress-induced detrusor overactivity (Fig. 1).

Cold stress-induced detrusor overactivity and skin surface temperature

As measured with a digital thermography camera, within 5 - 10 min after transfer to LT, the hind leg skin surface temperature of the rats decreases in correlation with the cold stress-induced detrusor overactivity (Fig. 2d). By the end of LT Phase I (20 min) and through Phase II (40 min), the skin temperature is relatively stable (Fig. 2d). Thus, while skin temperature has become stable by 20 min of LT and is maintained by for the duration of exposure, the cold stress-induced detrusor overactivity gradually disappears. The time-dependent reductions of LT stimulated-responses represent an adaptive response that is universal in normal healthy humans. Thus, a momentary cold stimulus can act as a trigger for the urinary responses, and our results suggest that the cold stress-induced detrusor overactivity is associated with the resulting sudden decrease of skin temperature.[13] After regaining homeostasis of skin temperature, even at levels significantly lower than they are at RT, the cold responses diminish. Therefore, the decrease of skin temperature is closely associated with the cold stress-induced detrusor overactivity (Fig. 1).

TRPM8 channels

We focused on the mammalian transient receptor potential (TRP) channel expression in the skin because the cold stress-induced detrusor overactivity is correlated with skin surface temperature. The mammalian TRP channel family consists of 28 channels that are subdivided into 5 different classes: TRPV (vanilloid), TRPC (canonical), TRPM (melastatin), TRPML (mucolipin), and TRPA (ankyrin).[19] TRP channels can be activated by physical (voltage, mechanical, heat, or cold) stress or chemical (pH, osmolality) stimuli.[20-24] TRPM8 can be activated by both menthol and LT stimuli (less than 25-28^{o}C).[25-28] The cell bodies of temperature sensitive neurons, consisting mostly of C- and Aδ-fibers, are located in both the trigeminal ganglia and dorsal root ganglia (DRG). TRPM8 channel protein and mRNA are found in about 10-15% of the trigeminal ganglia and 5-10% of the DRG.[29-31] TRPM8 channel knockout mice, which are not different from wild-type mice in overall appearance, general behavior, viability, core body temperature, or anatomy of the sensory ganglia, have significantly less cold sensitivity and almost no menthol sensitivity.[32-33] In addition, the TRPM8-expressing sensory neurons with dichotomizing axons within DRG may have a

role in urinary urgency evoked by cold sensation.[34] Therefore, we focused on correlations between cold stress-induced detrusor overactivity and TRPM8 channels.

Functional roles of TRPM8 channels expressed within the skin in cold stress-induced detrusor overactivity

The TRPM8 channel agonist menthol elicits detrusor overactivity in healthy, conscious rats (Fig. 1).[9,10] Spraying a liquid stream of 90% menthol solution once every 5 min for 20 min at RT induces detrusor overactivity patterns that significantly decreased voiding interval (Fig. 3a), micturition volume, and bladder capacity (Fig. 3b). The micturition patterns are very similar to the patterns of cold stress-induced detrusor overactivity that occur immediately after LT exposure. The basal pressures of menthol-sprayed rats tend to increase, though the increases are not statistically significant. In contrast, the basal pressures of the cold stress-exposed rats significantly increase. The difference in response may be due to the nature of local stimulation by menthol spray and whole body stimulation by LT.[9,10]

To investigate roles of TRPM8 channels in the menthol spray- and cold stress-induced detrusor overactivity mechanism(s), we used a TRPM8 channel antagonist, N-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl)piperazine-1-carboxamide (BCTC).[10] In rats intravenously injected with 0.1 μmol/kg BCTC, the menthol spray-induced detrusor overactivity is completely blocked.[10] After menthol spraying, voiding interval (Fig. 3a) and bladder capacity (Fig. 3b) of BCTC-treated rats do not decrease. In the BCTC-treated rats, the cold stress-induced detrusor overactivity that immediately occurs after transfer to LT is also partially abolished.[10] Additionally, the decreases of voiding interval (Fig. 3c), micturition volume, and bladder capacity (Fig. 3d) are inhibited in a dose-dependent manner.

Another cold-sensitive TRP channel, TRPA1, is important in the transduction of cooling sensations that are activated by noxiously cold temperatures of less than 17ºC.[35-39] However, the TRPA1 channel agonist, cinnamon, does not elicit detrusor overactivity that is similar to the menthol-induced detrusor overactivity.[9] While BCTC has a blocking effect for another thermosensitive channel, TRPV1 channels, the channels expressed in the skin are not likely to be functionally associated with the cold

stress-related detrusor overactivity. TRPV1 is present in the skin, but it is responsive only to temperatures that are greater than 43ºC.[19] Our results suggest that both menthol spray- and cold stress-responses are, in part, mediated through the neurological pathway involving TRPM8 channels (Fig. 1).[9,10] Consequently, the evidence suggests that TRPM8 channels expressed in the skin act as triggers for cold stress-induced detrusor overactivity (Fig. 1).

Functional roles of α1-adrenergic receptors in cold stress-induced detrusor overactivity

In addition to the RTX-sensitive C-fiber sensory nerve pathway, the cold stress-induced detrusor overactivity is partially mediated through the sympathetic nervous system that is also associated with transient hypertension. Thus, we have focused on α1-ARs that are activated by epinephrine and increase vascular tone, and consequently, blood pressure. α1-ARs are present at parasympathetic nerve terminals in the urinary bladder[40,41] as well as in the bladder urothelium where they mediate afferent bladder activity.[42-45] Therefore, we investigated the role of α1-ARs in cold

stress-induced detrusor overactivity by using the α1-AR antagonists naftopidil (high affinity for α1D-AR), silodosin (high affinity for α1A-AR), tamsulosin (affinity for α1A/1D-AR), and prazosin (non-selective α1-AR).[11]

All of the α1-AR antagonists partially mitigate cold stress-induced detrusor overactivity.[11] For instance, reductions in voiding interval (Fig. 4a) and bladder capacity (Fig. 4b) after transfer to LT conditions (Phase I) are inhibited in a dose-dependent manner by the antagonists. Except for prazosin, the antagonists prevent the increase in blood pressure that routinely occurs upon exposure to the LT environment.[11] Therefore, cold stress-induced detrusor overactivity that occurs simultaneously with increasing blood pressure is mediated, at least in part, by stimulation of the α1-AR subtypes α1D-AR and α1A-AR.[11,12] Our results also suggest that administration of α1-AR antagonists have the potential to treat bladder storage symptoms such as urinary frequency and nocturia that are exacerbated by cold stress (Fig. 1).

Resistance to the cold stress responses provided by traditional Japanese herbal mixtures

To treat for cold stress-exacerbated LUTS, we were interested in Japanese herbal mixtures. HARNCARE® (THC-002, TAIHO Pharmaceutical Co., Ltd., Tokushima, Japan), TJ-107® (Goshajinkigan, Tsumura Co., Tokyo, Japan) are produced from the traditional Japanese herbal mixture, Hachimijiogan. THC-002 is a galenical solution extracted from Hachimijiogan and refined by removal of the starch component. After 7 days of administering THC-002 by stomach tube, skin temperature of the treated rats is significantly increased compared to the THC-002-free control rats at RT.[13] While the skin temperature of THC-002 treated rats tends to be higher than that of the control rats during LT exposure, THC-002 does not completely prevent the decrease in skin temperature.[13] Compared to control rats (Fig. 5a), THC-002 partially inhibits the cold stress-induced detrusor overactivity associated with increased micturition frequency and decreased micturition volume (Fig. 5b). Additionally, TRPM8 channel protein (Figs. 5c, d) and mRNA expression levels within the skin of THC-002-treated rats significantly decrease.[13] THC-002 and Goshajinkigan both mitigate the C-fiber sensory nerve pathway-related detrusor overactivity by decreasing the tachykinin neurotransmitters and/or receptors related to C-fiber activation in the urinary bladder[46-49] and urethra.[50] In

future studies, we will investigate the mechanism(s) of increased skin temperature and decreased TRPM8 expression levels within the skin by administration of THC-002. At present, we simply report the empirical observation that some traditional Japanese herbal mixtures effectively mitigate detrusor overactivity elicited by cold stress (Fig. 1).

Some people without clinically evident cardiovascular disease experience cool hands and/or legs, even in a warm environment.[8] These sensations are an indication of cold sensitivity, which is an important concept in oriental medicine. People having cold sensitivity show high LUTS storage symptoms compared to people who are not sensitive to cold.[8] In addition, some cold-sensitive patients tend to complain about a change for the worse of their symptoms during cold periods. Some galenicals produced from traditional Japanese herbal mixtures can reduce the sensitivity to cold,[51,52] possibly by improving blood circulation.[53] While we did not investigate changes of blood circulation or protein factors associated with improved circulation, our clinical studies show that treatment by THC-002 can reduce cold sensitivity and mitigate LUTS storage symptoms.[8] Therefore, the traditional Japanese herbal mixtures, mainly composed of

Hachimijiogan, have the potential to provide resistance to cold stress-exacerbated LUTS.

Advantages and disadvantages in cold stress-induced detrusor overactivity models

To investigate LUTS, many researchers have strived to develop disease models, utilizing chemical irritants or physical destruction of tissues and/or pharmacological techniques. However, these models do not necessarily reflect normal disease processes. Our cold stress model seriously affects physical changes in basal metabolism, blood circulation, and/or peripheral nerve systems and induces detrusor overactivity in healthy rats without disease or injury. In addition, the model imitates micturition patterns that simulate human physiological responses due to sudden drops to low temperatures.

Many disease animal models are used to investigate LUTS, such as spontaneously hypertensive, diabetic, or ovariectomized rats. We often find that the lower urinary tract dysfunctions of these animals vary greatly among individuals at RT. However, our cold stress model mitigates the variability that is due to intrinsic and/or potential lower urinary tract dysfunctions. Recently, we showed that the ovariectomized rats exhibit

detrusor overactivity that is greater than that in sham-operated rats when exposed to LT, while the differences between ovariectomized and sham-operated rats at RT are minimal.[12] Therefore, the advantages of our model are useful to investigate the mechanisms of pathogenesis, aggravation, and/or treatment of LUTS (Fig.1).

In spite of these advantages, our model has two disadvantages. One is that it reflects systematic reactions due to “whole body” cooling. Thus, the analysis of partial reactions and changes in various receptors, neurotransmitters, and/or enzymes is limited (Fig. 1). Another is that our model provides only temporary and instantaneous stimulation, but not repeated and/or extended periods of cold stimulation. For patients having LUTS, repeated and/or continued cold environmental stress is the most serious problems. To overcome these disadvantages, we need more precise and detailed investigations.

Conclusions

This review supports the validity of using conscious healthy rats as a model for investigating cold stress-exacerbated LUTS that is associated with detrusor overactivity

(Fig. 1). RTX-sensitive C-fiber sensory nerve pathways involving α1-ARs and TRPM8 channels in skin cells and/or sensory neurons mediate the cold stress-induced detrusor overactivity. In addition, decreases of skin temperature correlate with the detrusor overactivity. α1-AR antagonists and traditional Japanese herbal mixtures composed of Hachimijiogan have the potential to treat or prevent cold stress responses that can exacerbate LUTS. Therefore, our cold stress-detrusor overactivity model will be useful in the investigation of the mechanism(s) of pathogenesis, treatment, and/or prevention for LUTS. Furthermore, our future studies of the cold stress response will explore changes in animal models that mimic the effects of human aging, metabolic disturbances, altered peripheral circulation, and nervous system dysfunctions. Based on the outcome of these studies, we feel that novel combinations of drugs can be developed that will provide new insights into the onset and treatment of urinary cold stress responses.

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Figure Legends

Fig. 1 Our projects and findings in cold stress-exacerbated LUTS. This review summarizes the functional relationship of cold stress-exacerbated LUTS with unmyelinated C-fiber sensory nerve pathways (red lines) and TRPM8 channels in skin. In addition, α1-AR antagonists, TRPM8 channel antagonist, and traditional Japanese herbal mixtures have the potential to treat cold stress-exacerbated LUTS (green arrows). Our model has limitations due to "whole body" cooling (red boxes with question marks). The analysis of partial reactions and changes in various receptors, neurotransmitters, and/or enzymes are limited (dotted boxes with black question marks. We have not determined which direct or indirect mechanisms mediate cold stress-induced LUTS through TRPM8 channels (red line arrows) or skin temperature (red dotted line arrows). Our future projects will investigate the cold stress response by using animal models for aging, hypertension, or diabetes (red question marks) to provide new insights of LUTS. Furthermore, we will investigate existing and/or novel drugs (green question marks) to develop treatments for LUTS.

Fig. 2 Cold stress-induced detrusor overactivity. (a) Healthy conscious rats transferred from RT (27±2ºC) to LT (4±2ºC) exhibit detrusor overactivity patterns including increased micturition frequency during Phase I of LT exposure. Later, during Phase II, the detrusor overactivity patterns slowly mitigate and disappear so that little further change occurs upon return to RT. Arrowheads: transfer between RT and LT. (b and c) Voiding interval (b) and bladder capacity (c) of non-treated rats (white) transferred to LT significantly decreased. However, the decreases of both values in RTX-treated rats (black) are partially inhibited compared to the non-treated rats. *P<0.05, **P<0.01 compared to RT; †P<0.01, compared to Phase I; §P<0.01, compared to Phase II. (d) After transferring to LT, blood pressure (squares) significantly increases compared to RT. The increased blood pressure is maintained through the LT exposure period. After transferred to LT, the skin temperature (circles) significantly decreases. At 20, 30, and 40 min under LT, the skin temperatures are relatively stable.

Fig. 3 Effects of the TRPM8 channel antagonist BCTC on menthol- and cold stress-induced detrusor overactivity. (a and b) Voiding interval (a) and bladder capacity

(b) of the rats sprayed with menthol decreases significantly. The rats treated with 0.1 μmol/kg BCTC do not exhibit decreases due to the menthol spray. *P<0.01, compared to pre-menthol spraying. (c and d) BCTC inhibits in a dose-dependent manner decreases of voiding interval (c) and bladder capacity (d) that are induced by cold stress. *P<0.01, compared to RT.

Fig. 4 Effects of α1-AR antagonists on cold stress-induced detrusor overactivity. (a and b) α1-AR antagonists inhibit in a dose-dependent manner the decreased voiding interval (a) and bladder capacity (b) that occur immediately after transfer to LT. *P<0.01, compared to RT.

Fig. 5 The traditional Japanese herbal mixture, THC-002, provides resistance to cold stress-exacerbated LUTS. (a and b) Compared to THC-002-free control rats (a), the cold stress-induced increases in micturition frequency and decreases micturition volume are partially inhibited in THC-002-treated rats (b). Arrowheads: transfer from RT to LT. Immunofluorescent intensity of TRPM8 channels within the subcutaneous tissue of the

left hind leg skin in control rats (c) is stronger than that in the THC-002-treated rats (d). Red (arrows), TRPM8 channel-positive areas; Green, tubulin beta 3-positive peripheral nerve fibers. Blue, nuclei.

Abbreviations & Acronyms

LUTS = lower urinary tract symptoms

RTX = resiniferatoxin

TRPM8 = transient receptor potential melastatin 8

AR = adrenergic receptor

RT = room temperature

LT = low temperature

DRG = dorsal root ganglia

BCTC = N-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl)piperazine-1-carboxamide

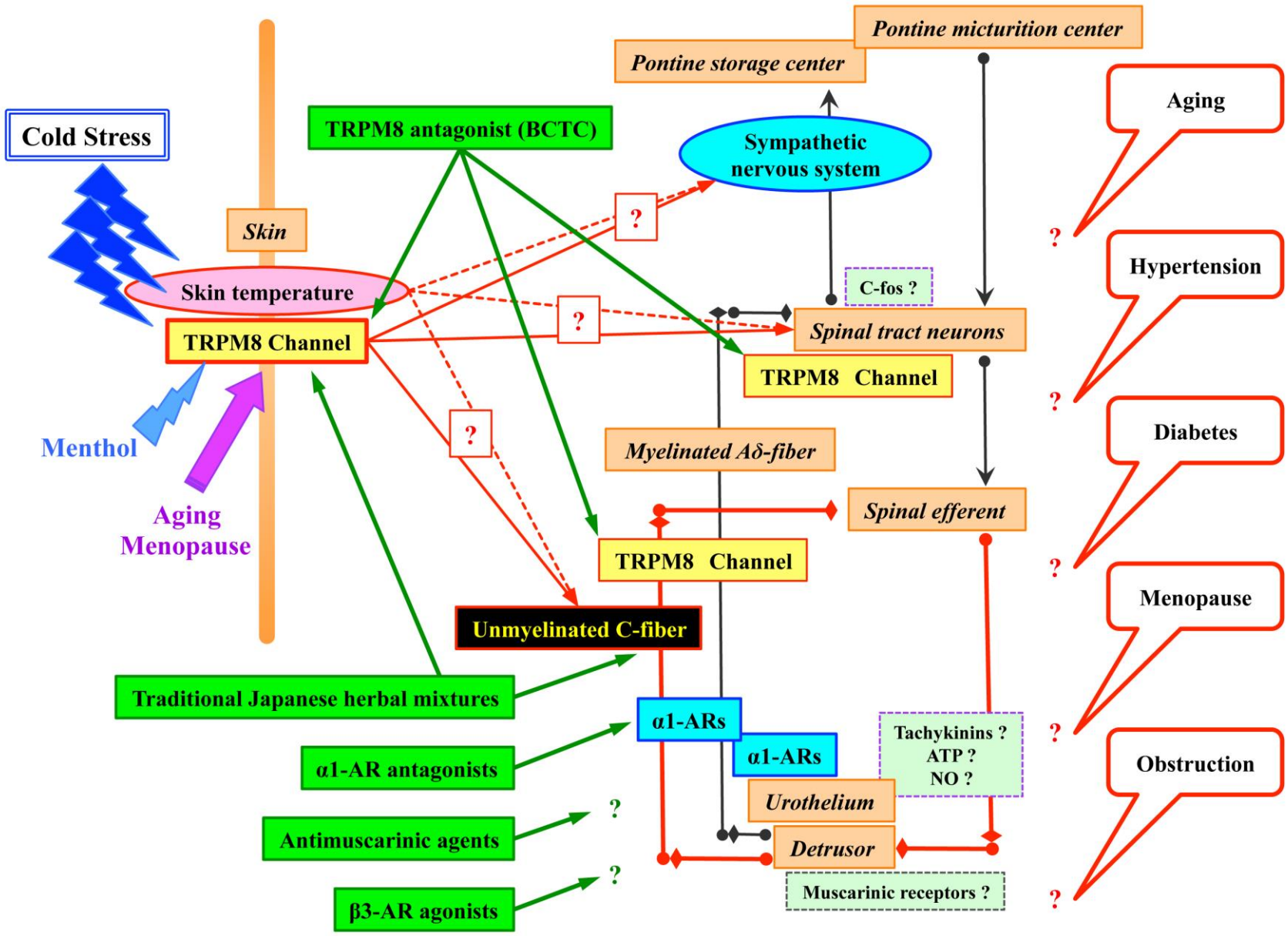
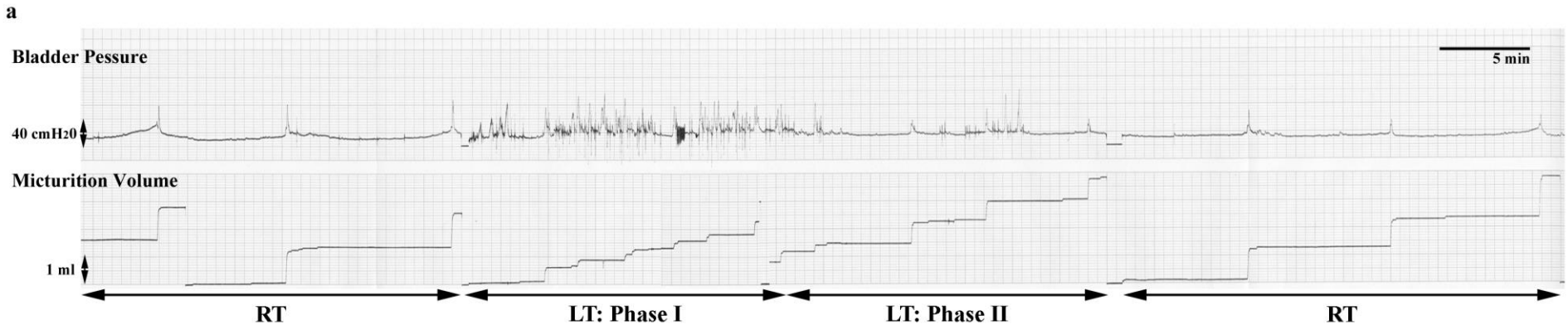
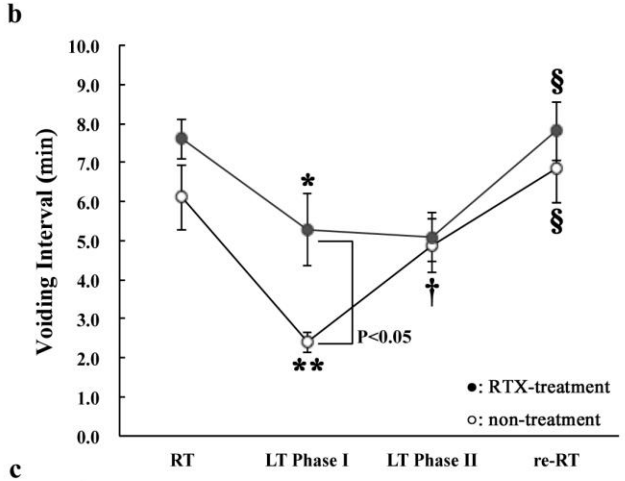
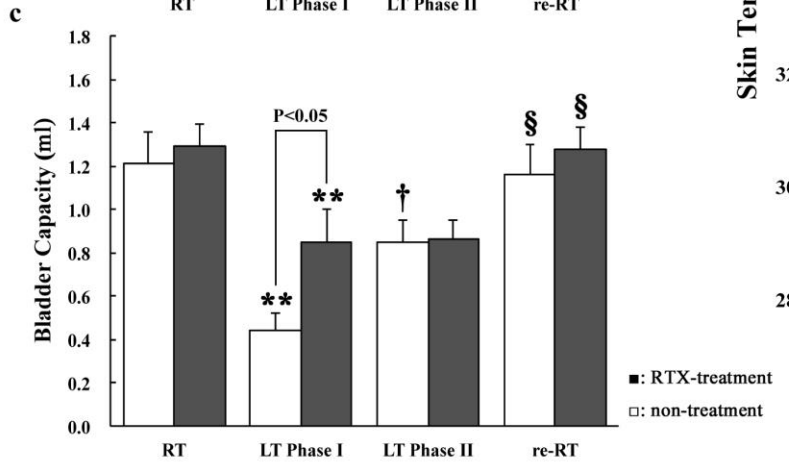

Cold Stress
Skin
Skin temperature
TRPM8 Channel
Menthol
Aging
Menopause
TRPM8 antagonist (BCTC)
Traditional Japanese herbal mixtures
α1-AR antagonists
Antimuscarinic agents
β3-AR agonists
Pontine micturition center
Pontine storage center
Sympathetic nervous system
C-fos ?
Spinal tract neurons
TRPM8 Channel
Myelinated Aδ-fiber
Spinal efferent
TRPM8 Channel
Unmyelinated C-fiber
α1-ARs
α1-ARs
Tachykinins ?
ATP ?
NO ?
Urothelium
Detrusor
Muscarinic receptors ?
Aging
Hypertension
Diabetes
Menopause
Obstruction

Fig. 1


a
Bladder Pessure
5 min
40 cmH20
Micturition Volume
1 ml
RT
LT: Phase I
LT: Phase II
RT


b
Voiding Interval (min)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
**
†
§
§
P<0.05
●: RTX-treatment
○: non-treatment
RT
LT Phase I
LT Phase II
re-RT


c
Bladder Capacity (ml)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
P<0.05
**
**
†
§
§
■: RTX-treatment
□: non-treatment
RT
LT Phase I
LT Phase II
re-RT

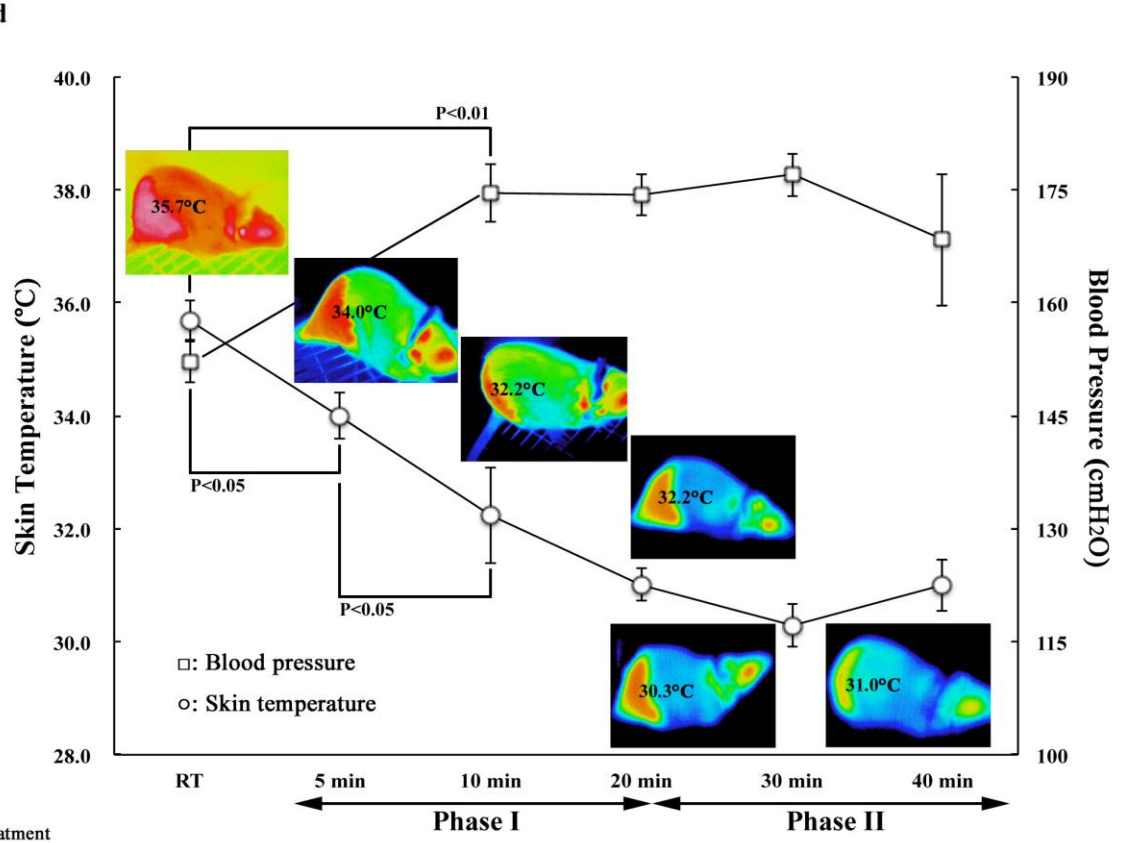

d
Skin Temperature (°C)
40.0
38.0
36.0
34.0
32.0
30.0
28.0
Blood Pressure (cmH2O)
190
175
160
145
130
115
100
P<0.01
P<0.05
P<0.05
35.7°C
34.0°C
32.2°C
32.2°C
30.3°C
31.0°C
□: Blood pressure
○: Skin temperature
RT
5 min
10 min
20 min
30 min
40 min
Phase I
Phase II

Fig. 2

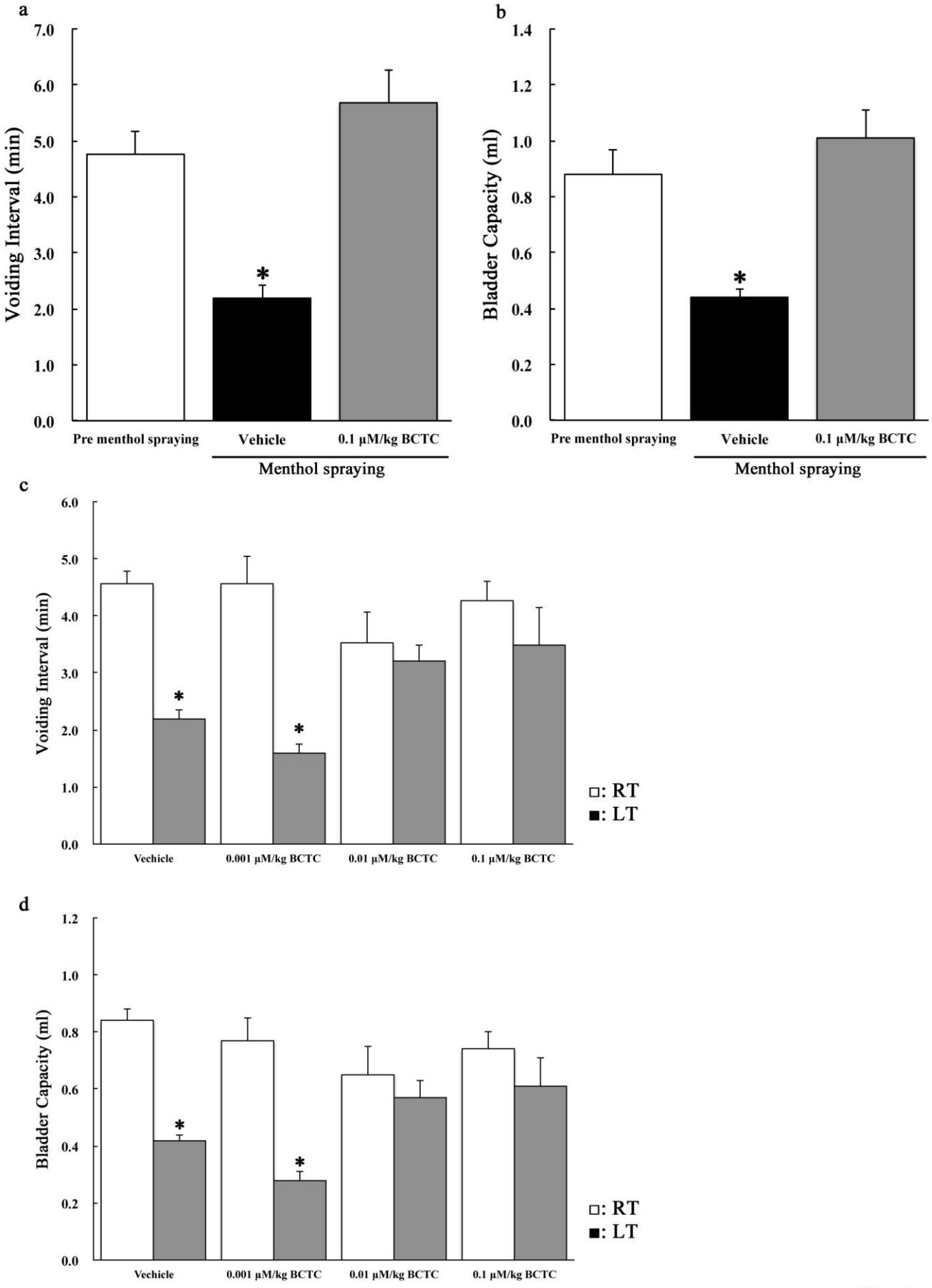

a
Voiding Interval (min)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
Pre menthol spraying
Vehicle
0.1 μM/kg BCTC
Menthol spraying
b
Bladder Capacity (ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Pre menthol spraying
Vehicle
0.1 μM/kg BCTC
Menthol spraying
c
Voiding Interval (min)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
*
Vechicle
0.001 μM/kg BCTC
0.01 μM/kg BCTC
0.1 μM/kg BCTC
□: RT
■: LT
d
Bladder Capacity (ml)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Vechicle
0.001 μM/kg BCTC
0.01 μM/kg BCTC
0.1 μM/kg BCTC
□: RT
■: LT

Fig. 3

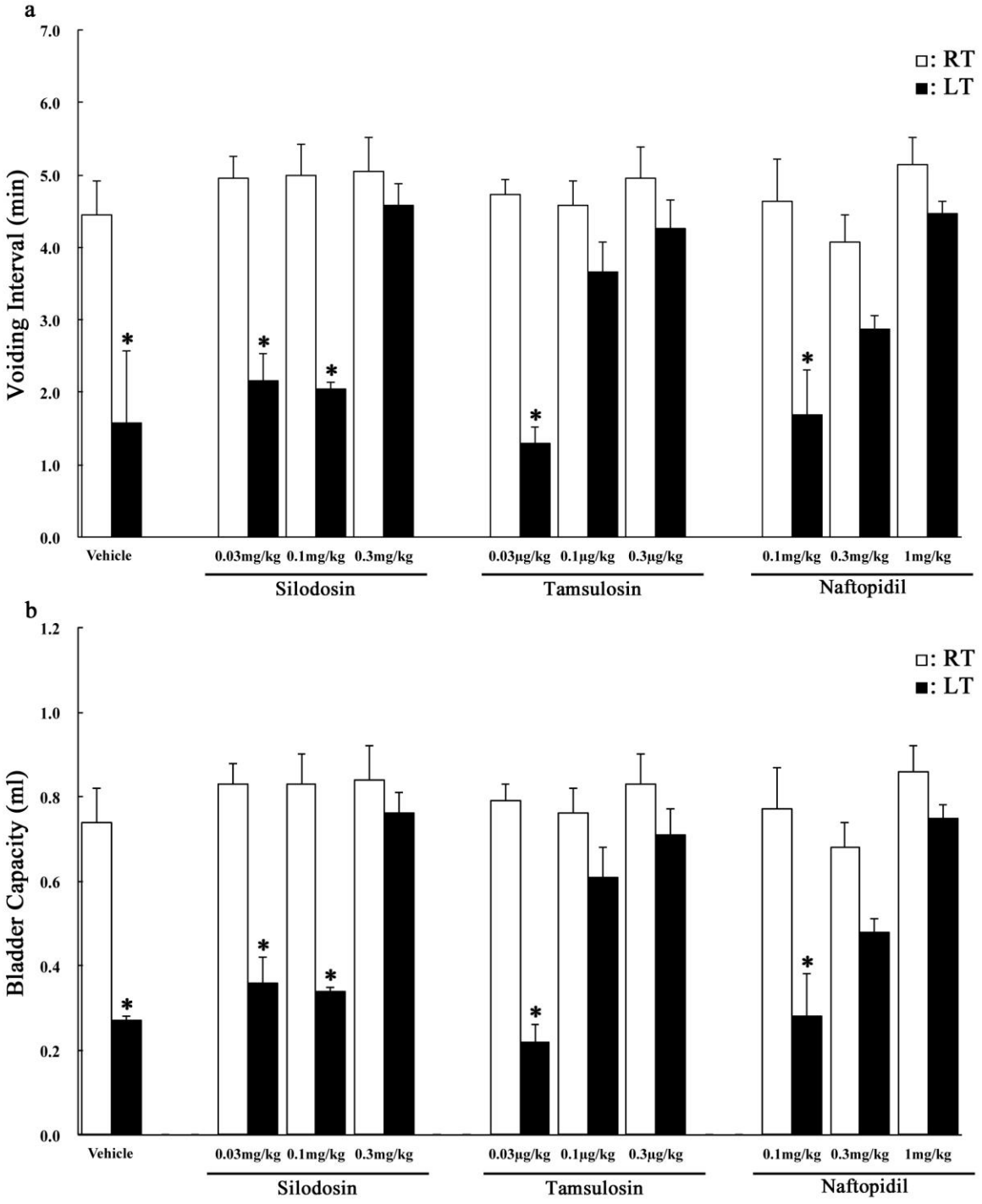

a
Voiding Interval (min)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
□: RT
■: LT
*
Vehicle
0.03mg/kg 0.1mg/kg 0.3mg/kg
Silodosin
0.03μg/kg 0.1μg/kg 0.3μg/kg
Tamsulosin
0.1mg/kg 0.3mg/kg 1mg/kg
Naftopidil
b
Bladder Capacity (ml)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
□: RT
■: LT
*
Vehicle
0.03mg/kg 0.1mg/kg 0.3mg/kg
Silodosin
0.03μg/kg 0.1μg/kg 0.3μg/kg
Tamsulosin
0.1mg/kg 0.3mg/kg 1mg/kg
Naftopidil

Fig. 4

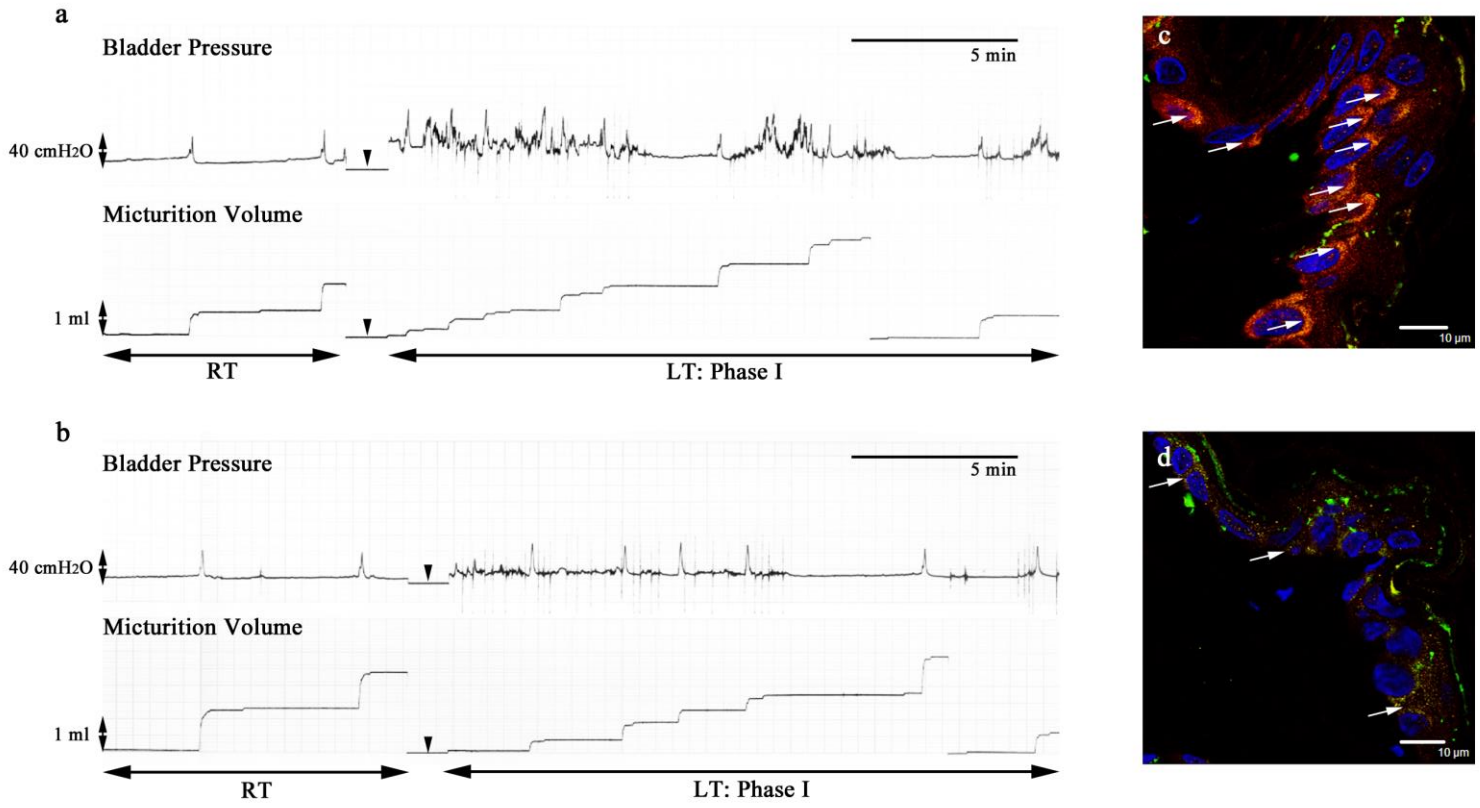

a
Bladder Pressure
5 min
40 cmH2O
Micturition Volume
1 ml
RT
LT: Phase I
b
Bladder Pressure
5 min
40 cmH2O
Micturition Volume
1 ml
RT
LT: Phase I
c
10 μm
d
10 μm

Fig. 5