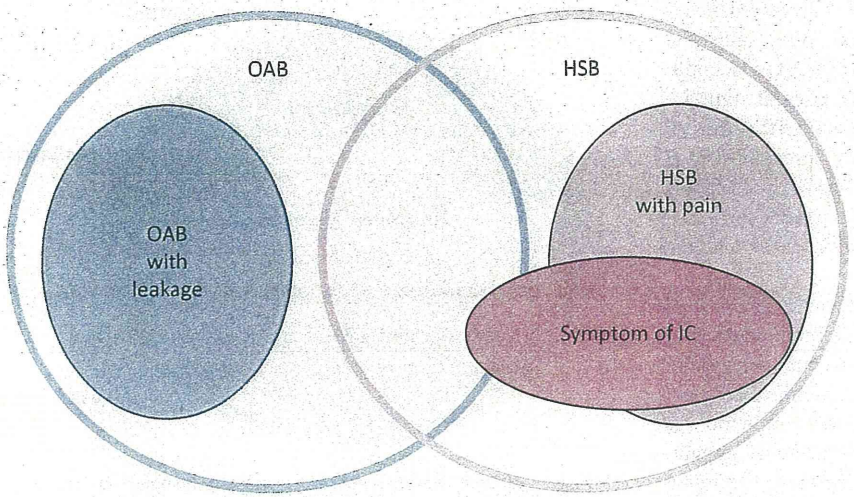

Fig. 2 Relationship between HSB and its related terms. Symptoms of OAB (urinary urgency, usually associated with urinary frequency and nocturia, with or without urgency incontinence) and HSB (increased bladder sensation, usually associated with urinary frequency and nocturia, with or without bladder pain) are overlapping, although OAB with leakage and HSB with pain are readily separated. Symptoms of IC usually present HSB symptoms, often accompanying pain, but occasionally mimicking OAB symptoms.

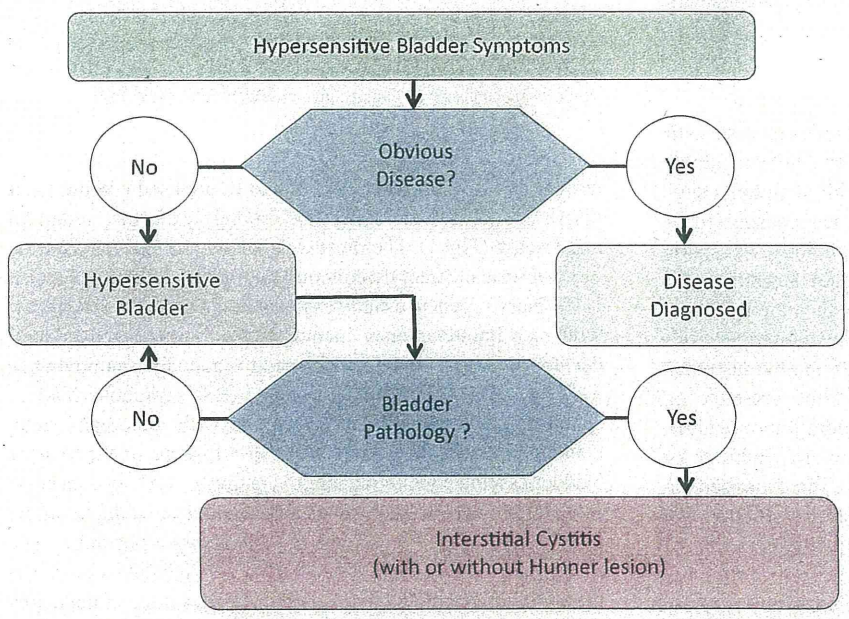

Fig. 3 Algorithm for hypersensitive bladder symptoms. HSB symptoms should be investigated for the causes. When there is no obvious disease, the condition is called as HSB. When bladder pathology is identified in HSB, the diagnosis of IC is made. When there is no such bladder abnormality, the condition remains as HSB. The presence or absence of Hunner lesion should be recorded for IC cases.

In contrast, IC should be defined by three conditions: HSB symptoms, bladder pathology, and no other diseases explaining the symptoms. Bladder pathology shows Hunner's lesion or glomerulations after hydrodistention, if following NIDDK criteria.[9] However, the specificity of glomerulations is challenged and needs further investigation. Tentatively, by allowing both findings for bladder pathology, we should describe the presence of absence of Hunner's lesion for each patient for the future analysis.

HSB can be used as a diagnostic name for the condition that is suspected of IC, but has not fulfilled the requirements for IC diagnosis. HSB symptoms might occur with or without evidence for bladder pathology. It could accompany chronic pelvic pain or functional disorders. By analogy, OAB can be used as a diagnostic name for the condition that is suspected of detrusor overactivity, but has not fulfilled the requirements for DO diagnosis. OAB symptoms might occur with or without evidence for detrusor overactivity. It could accompany benign prostatic enlargement or stress urinary incontinence.

The relationship between HSB and related terms, IC and OAB, is shown in Fig. 2. Symptomatically, HSB and OAB are substantially overlapping. Detailed voiding recording with reference to bladder sensation indicated increased urinary sensation at a given bladder volume in OAB.[40,41] Urodynamically, those diagnosed as OAB have shown more detrusor overactivity and lower bladder volume at urinary sensation and increased sensitivity to electrostimulation.[42] These observations suggest OAB symptoms also reflect hypersensitivity of the bladder. IC is a representative disease causing HSB symptoms, most typically with pain, but might be painless and indistinguishable from OAB.

An algorithm for hypersensitive bladder symptoms is shown in Fig. 3. When there is no obvious disease explaining HSB symptoms, the condition is called HSB. When bladder pathology is identified in HSB, the diagnosis of IC is made. When there is no such bladder abnormality, the condition remains as HSB. Bladder pathology is either Hunner's lesion or glomerulations after hydrodistention, although the latter is to be

challenged for specificity. The presence or absence of Hunner's lesion should be recorded to characterize IC cases.

With these definitions, the following makes sense: (i) a woman complaining of HSB symptoms proved to have carcinoma *in situ* of the bladder; (ii) a woman with HSB symptoms was diagnosed with HSB because no obvious diseases were apparent, but now she is diagnosed with IC based on cystoscopic abnormality; and (iii) a man with HSB showed no abnormal findings at hydrodistention, so he remained as HSB.

Introducing HSB, the counter concept of OAB, into bladder dysfunction taxonomy will clarify current confusion surrounding IC, and importantly lessen the ignorance of IC by the medical society.

Conflict of interest

None declared.

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Neurourology and Urodynamics

Effects of L-Arginine, Mirabegron, and Oxybutynin on the Primary Bladder Afferent Nerve Activities Synchronized With Reflexic, Rhythmic Bladder Contractions in the Rat

Naoki Aizawa,[1] Yukio Homma,[2] and Yasuhiko Igawa[1]*
[1]Department of Continence Medicine, The University of Tokyo Graduate School of Medicine, Tokyo, Japan
[2]Department of Urology, The University of Tokyo Graduate School of Medicine, Tokyo, Japan

Aims: We measured single-unit mechanosensitive afferent activities (SAAs) during reflexic, rhythmic bladder contractions (RBCs), and examined whether L-arginine, an NO substrate, and mirabegron, a β3-adrenoceptor agonist, and oxybutynin, an antimuscarinic agent, can affect the SAAs in such condition. **Methods:** Twenty-nine female Sprague-Dawley rats were anesthetized. SAA was identified by electro-stimulation of the left pelvic nerve and by bladder distension, and was divided into Aδ- or C-fibers by conduction velocity. To produce the RBCs, right L6 dorsal roots were kept intact. Under an isovolumetric condition, vehicle and L-arginine (300 mg/kg) or mirabegron (1 mg/kg) or oxybutynin (1 mg/kg) were administered intravenously. **Results:** All of the Aδ- (n = 26) and C-fibers (n = 29) capable of responding to bladder distention were also responsive to bladder contractions during RBCs. The amplitude and duration of RBCs significantly decreased after mirabegron- and oxybutynin-administrations, but not after L-arginine-administration. The interval of RBC was significantly elongated after L-arginine- and mirabegron-administrations. Regarding the SAAs, the peaks of firing rate (FR) during RBCs and FR during the non-contractile phase were decreased after L-arginine-administration, which were more remarkable for Aδ-fibers than C-fibers. Similar results were observed after mirabegron-administration only for Aδ-fibers. After oxybutynin-administration, the peak of FR of both fiber-SAAs significantly decreased, but the change was not significant when the value was normalized by the amplitude of RBCs. **Conclusions:** The present results indicate that mechanosensitive Aδ- and C-fibers were also responsive to bladder contractions, and that NO production and β3-adrenoceptor stimulation can inhibit SAAs mainly of Aδ-fibers synchronized with RBCs. *Neurourol. Urodynam.* © 2014 Wiley Periodicals, Inc.

Key words: afferent; β3-adrenoceptor; nitric oxide; Sprague-Dawley rats; urinary bladder

INTRODUCTION

Reflexic bladder contractions begin when the afferent impulses conveyed by the pelvic afferent nerves reach the complex neural interactions of the central nerves system (CNS: pons, periaqueductal grey (PAG), brain frontal cortex, lumbosacral spinal cord).[1] Thus, excitation of the mechanoreceptors in sensory nerve endings plays an important role in triggering the micturition reflex. The afferent innervation of the urinary bladder consists primarily of small myelinated Aδ-fiber and unmyelinated C-fiber axons that respond to mechanical stimuli. In the pelvic nerve of cats, various authors[2,3] have described that mechanosensitive fibers (conduction velocities: CV = 1.7–2.5 m/sec) in the detrusor muscle layer, which may include Aδ-fibers, can be responded to distension but also to contractions of the bladder, suggesting that contractions and distension of the bladder activate the afferent endings by a common mechanism.

In the rat, there is evidence that many C-fibers (CV = less than around 2.0 m/sec) respond to slow distension of the bladder with physiological volumes: some of them are "volume" receptors that do not respond to contractions of the bladder.[4,5] Chuang et al. reported that in the rat, volume receptors are activated by bladder distension but do not fire in response to bladder contractions, whereas tension receptors located in the muscle layers deeper in the bladder wall respond to an isovolumetric bladder contraction as well as distension,[6] which is also observed similarly in the cat.

We have previously demonstrated that the increased endogenous nitric oxide (NO) and stimulation of β3-adrenoceptor (β3-AR) can inhibit single-unit afferent activities (SAAs) of mechanosensitive primary bladder afferent nerves during bladder distension in an in vivo rat model.[7–9] In that model, no reflexic bladder contractions are elicited by bladder distension, as bilateral L6 dorsal roots, the main afferent pathway from the bladder, have been dissected off. In the current study, in order to investigate the characteristics of SAAs capable of responding to bladder contractions, we developed a new model for measurement of SAAs synchronized with reflexic, rhythmic bladder contractions (RBCs), in which keeping the right L6 dorsal root intact although the left L6 dorsal root is resected for the afferent measurements, by modifying our previous SAAs-measurement model. By using this new model, we investigated whether an increase in endogenous NO production induced by L-arginine (an NO-substrate), mirabegron (a β3-AR agonist), and oxybutynin (an antimuscarinic agent), can affect the SAAs during reflexic RBCs.

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*Correspondence to: Yasuhiko Igawa, M.D., Ph.D., Professor and Chairman Department of Continence Medicine, The University of Tokyo Graduate School of Medicine, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: yigawa-jua@umin.ac.jp
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MATERIALS AND METHODS

Animals

Thirty-eight adult female Sprague-Dawley rats weighing 185–242 g (9–11 weeks old) were used (Nihon SLC, Shizuoka, Japan). The rats were maintained under standard laboratory conditions with a 12:12 hr light: dark cycle, and free access to food pellets and tap water. The protocol was approved by the Animal Ethics Committee of The University of Tokyo Graduate School of Medicine and in line with NIH guidelines for the care and use of experimental animals.

Surgical Procedure for the SAAs Measurements

The rats were anesthetized with urethane (1.2 g/kg intraperitoneally). Body temperature was maintained by a heated blanket at 38°C. The left pelvic nerve was dissected from surrounding tissue proximal to the major pelvic ganglion. A pair of silver electrodes was placed around the pelvic nerve. A polyethylene catheter (Clay-Adams PE-50, Becton Dickinson and company, Parsippany, NJ) was inserted in the bladder through the dome. Fine filaments were dissected from the left L6 dorsal root and placed across shielded bipolar silver electrodes. Clearly different unitary action potentials of afferent fiber originating from the bladder were identified by electrical stimulation of the pelvic nerve and bladder distention with saline. These action potentials were discriminated by the Spike2 (CED, Cambridge, UK) impulse shape recognition program. CV was calculated from the latency of response to electrical stimulation and the conduction distance between stimulation and recording sites, which was based on our anatomical data. Fibers were grouped based on CV. Those with a CV < 2.5 m/sec were considered to correspond to unmyelinated C-fibers and those with CV ≥ 2.5 m/sec to myelinated Aδ-fibers.[10] After detecting and classifying these mechanosensitive afferent activities, the following experiment was performed.

Experimental Protocol of the SAAs Synchronized With RBCs

To produce the RBCs, right L6 dorsal roots were kept intact and the urethral meatus was clamped at the level of the urethral meatus by forceps. After the bladder had been emptied, saline was instilled into the bladder at a rate of 0.16 ml/min. Bladder pressure and SAAs were recorded continuously on the Spike2 program. When RBCs appeared, the instillation was stopped and the bladder was kept under an isovolumetric condition. After RBCs appeared reproducibly for a period of 5 min, vehicle was administrated, and then L-arginine (300 mg/kg) or mirabegron (1 mg/kg) or oxybutynin (1 mg/kg) was administrated. The parameters of the bladder pressure and SAAs were analyzed during three or four of RBCs before and after the vehicle- and drug-administrations.

Following cystometric and SAA parameters were analyzed and represented as numeric-values or % of baseline (before administration) values: amplitude (cmH_2O) and duration (sec) of contraction; interval between contractions (sec); bladder basal pressure (cmH_2O); time to first peak (TFP) and time to first response (TFR) of contraction after vehicle- or drug-administrations (sec); mean firing rate (FR) during non-contractile phase (Hz); peak FR of SAA activity (Hz) and FR/bladder pressure (Hz/cmH_2O) during contraction, which value of FR/bladder pressure was used for normalization with change in amplitude of RBCs.

Drugs

N,N-Dimethylacetamide (DMA), L-arginine, and oxybutynin were purchased from Sigma-Aldrich (St. Louis, MO). Cremophor was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Mirabegron, (*R*)-2-{2-aminothiazol-4-yl)-4′-{2-[(2-hydroxy-2-phenylethyl)amino]ethyl} acetanilide, was provided from Astellas Pharma Inc. (Tokyo, Japan). L-arginine and oxybutynin were dissolved in saline. Mirabegron was dissolved in 5% DMA and 5% cremophor, and 90% distillated water. Drugs and vehicle were administrated intravenously through a polyethylene catheter (PE-50) placed in the right external jugular vein.

Statistical Analysis

All data are expressed as mean ± standard error of mean (S.E.M.). Results for two group comparisons were analyzed using paired Student's *t*-test. Results for multiple comparisons were analyzed using two-way ANOVA followed by Tukey's test. *P*-values < 0.05 are considered statistically significant.

RESULTS

Characteristics of SAAs Synchronized With RBCs

Totally 50 rats were subjected to induction of stable RBCs under an isovolumetic condition, but 12 animals (approximately 24%) failed to show stable RBCs, and these animals were excluded. Finally 38 rats (approximately 76%) were used for the present study, and average volume of saline instilled intravesically for inducing stable RBCs were 0.67 ± 0.04 ml (range: 0.31–1.23 ml). Bladder distension induced RBCs. We sometimes faced difficulty to keep an ideal isovolumetric condition for eliciting RBCs continuously. Thus we needed to do experiments within a short term period when RBCs were evoked continuously, which allowed us to evaluate three or four RBCs after vehicle- or drug-administrations. The shape of these RBCs was apparently different from that of "microcontractions" defined in our previous study[7] (Fig. 1). For example, the amplitude of the RBSs ranged 12–54 cmH_2O, and the corresponding value of microcontractions was 1.5–7 cmH_2O and was of myogenic origin as no reflex arc through the L6 dorsal roots was preserved.[7]

All of the both Aδ- and C-fibers that had responded to bladder distention were also responsive to bladder contractions during RBCs (Aδ-fibers: n = 33, CV: 8.14 ± 0.81 m/sec; C-fibers: n = 35, CV: 1.87 ± 0.41 m/sec).

At the beginning of the study for the drug-administrations, saline was administrated repeatedly to investigate the time-dependent influence on the bladder pressure parameters and SAAs of both fibers (Aδ- and C-fibers) throughout the experiments. As a result, none of the bladder pressure parameters (n = 9) and SAAs (Aδ-fibers: n = 7, C-fibers: n = 6) investigated changed significantly (data shown as the supplemental files).

Effects of L-Arginine, Mirabegron, and Oxybutynin on Bladder Pressure

The amplitude and duration of RBCs did not change either after vehicle- or L-arginine-administration. On the other hand, interval of RBCs, TFR, and TFP were significantly elongated after L-arginine-administration, although the basal pressure was decreased by vehicle-administration alone (Figs. Fig. 1A and 2A–D; and Table I). After mirabegron-administration, the amplitude and duration of RBCs significantly decreased

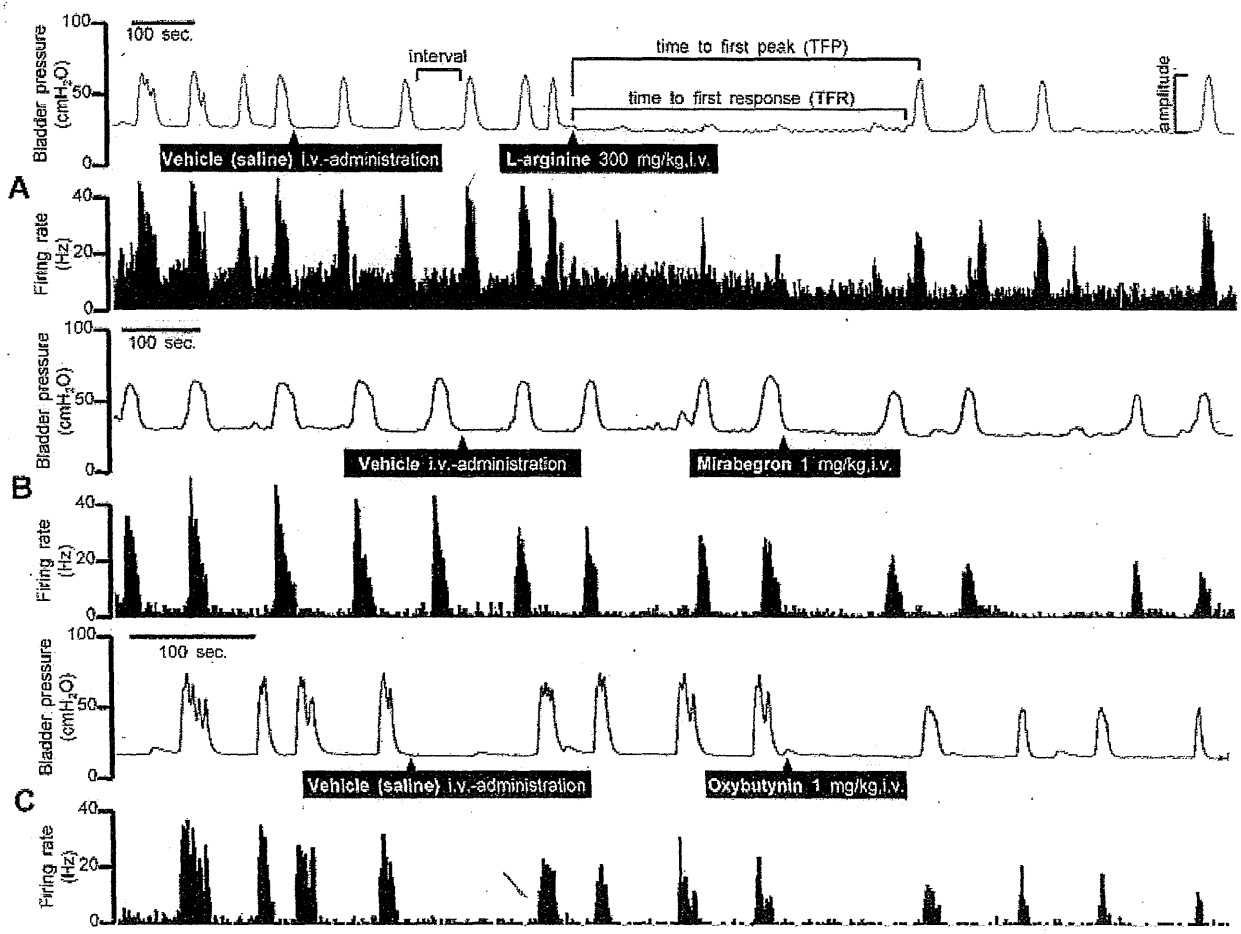

Fig. 1. Representative recordings of bladder pressure (upper tracings) and firing rate (lower tracings) during RBCs before and after vehicle- and drug-administrations. Moreover, several parameters were indicated in tracing A. **A**: Changes in the bladder pressure and firing rate of C-fiber with vehicle- and L-arginine-administrations. **B**: Changes in the bladder pressure and firing rate of Aδ-fiber with vehicle- and mirabegron-administrations. **C**: Changes in the bladder pressure and firing rate of C-fiber with vehicle- and oxybutynin-administrations.

(Figs. Fig. 1B and 2E, F). Similar effects were observed also after oxybutynin-administration (Figs. Fig. 1C and 2I, J). An elongation in RBCs-interval and a decrease in basal pressure were observed after mirabegron-administration but not oxybutynin-administration (Fig. 2G, H and K, L). There were no significant differences in TFR and TFP after either mirabegron- or oxybutynin-administration (Figs. 1 and 2; and Table I).

Effects of L-Arginine, Mirabegron, and Oxybutynin on SAAs Activities

After L-arginine-administration, the peak FR of SAAs and FR/bladder pressure values during RBCs and FR during the non-contractile phase were decreased, which were more remarkable in Aδ-fibers than C-fibers (Figs. Fig. 1A, 3A–C, and 4A–C). Similar results in Aδ-fibers, but not in C-fibers, were observed after

TABLE I. Numeric-Values of "TFR" and "TFP" After Vehicle- or Drug-Administrations

	Parameters (sec)	Vehicle-administration	Drug-administration	Numbers
L-arginine (300 mg/kg)	Time to first response (TFR)	82.67 ± 12.71	684.89 ± 148.54[a]	n = 9
	Time to first peak (TFP)	91.44 ± 13.35	725.44 ± 137.79[a]	
Mirabegron (1 mg/kg)	Time to first response (TFR)	68.77 ± 9.42	74.00 ± 15.29	n = 13
	Time to first peak (TFP)	80.54 ± 9.91	81.69 ± 15.52	
Oxybutynin (1 mg/kg)	Time to first response (TFR)	94.43 ± 17.43	66.43 ± 11.83	n = 14
	Time to first peak (TFP)	107.71 ± 17.69	76.14 ± 12.20	

The values are indicated as mean ± S.E.M.

[a]$P < 0.01$ significant difference from vehicle-administration (paired Student's *t*-test).

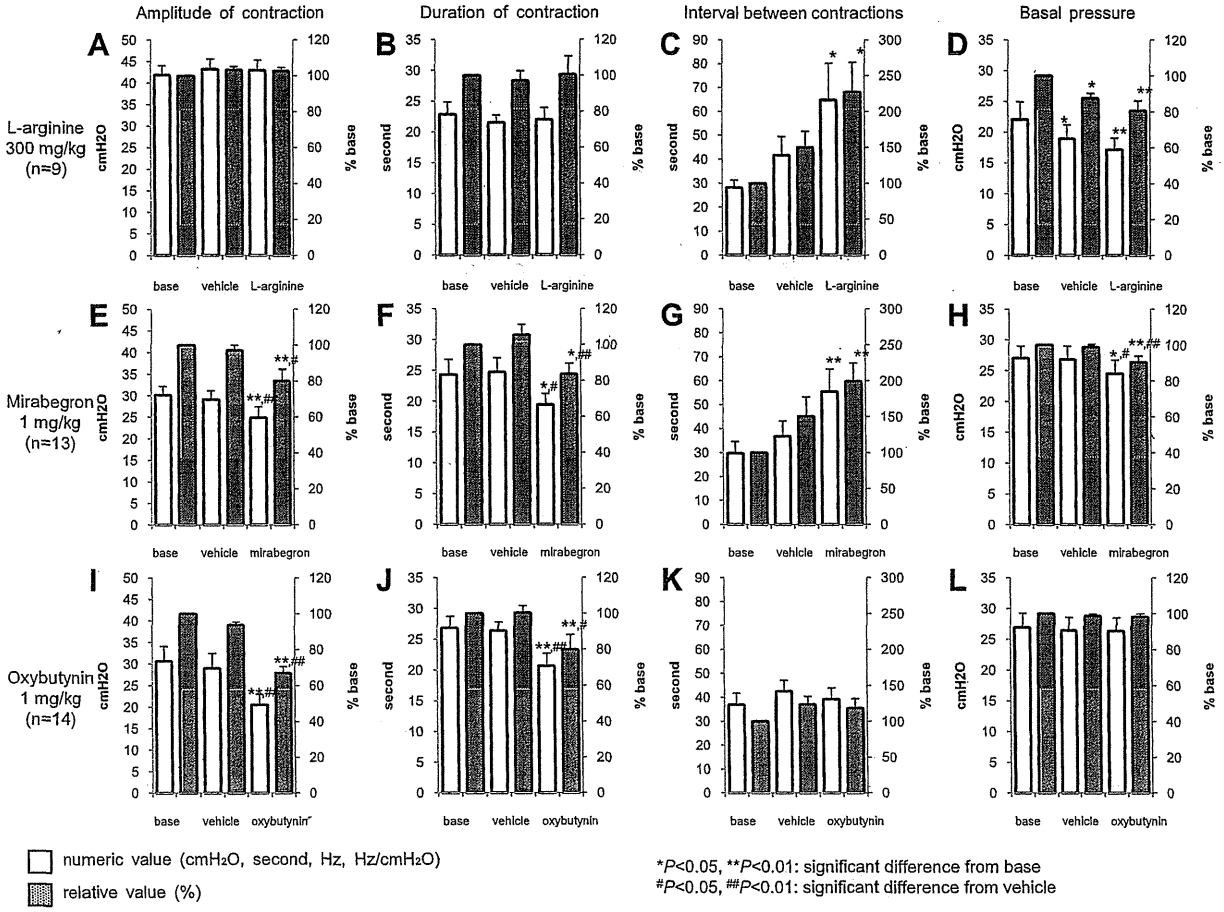

Fig. 2. Bladder pressure parameters before and after vehicle- and drug-administrations. **A–D**: L-arginine-administration. **E–H**: Mirabegron-administration. **I–L**: Oxybutynin-administration. White bars indicate the numeric-values (correspond to left Y-axis), and gray bars indicate the relative % values (correspond to right Y-axis).

mirabegron-administration (Figs. 1B, 3D–F, and 4D–F). After oxybutynin-administration, the peak FR of both fiber-SAAs significantly decreased, but the change was not significant when the value was normalized by the amplitude of RBCs (i.e., FR/bladder pressure during contraction) (Figs. Fig. 1C, 3G–I, and 4G–I).

DISCUSSION

In the present study, we have confirmed that all of the both Aδ- and C-fibers capable of responding to bladder distention (so-called mechanosensitive afferent fibers) were also responsive to bladder contractions during RBCs in the rat. In the present-experimental set-up, we have resected unilateral (left) L6 dorsal root for SAAs-measurement and preserved only the contralateral (right) L6 dorsal root, which may make RBCs under an isovolumetric bladder condition relatively weak and difficult to maintain continuously compared with the previous studies,[11–13] in which the micturition reflex arc has been fully preserved. Nevertheless in the present study, we could measure the SAAs during RBCs induced by bladder distension, which seems more physiological condition rather than RBCs induced by artificial procedure such as using drugs or electrical stimulation of the nerves. A previous study in the rat reported that tension receptors located in the deeper muscle layers of the bladder wall respond to isovolumetric bladder contractions as well as distension,[6] and Aδ-fibers are known to be located primarily within the detrusor smooth muscle layer.[14–17] The present findings obtained in Aδ-fibers, which showed that this class of fibers responded to bladder contractions but also to bladder distention, are in line with those previous findings.

In the present study, we have also confirmed that C-fibers capable of responding to bladder distention are also responsive to RBCs. In contrast, previous reports demonstrated that many afferents of C-fibers respond to slow distension of the bladder with physiological volumes: some of these are "volume" receptors that do not respond to contractions.[4–6] However, these previous studies did not investigate the direct SAAs of mechanosensitive C-fibers during the reflexic bladder contractions in an isovolumetric condition. Moreover, Shea et al. reported that there are many types of afferent fibers including Aδ- and C-fibers so-called mechano- or chemo-sensor individually and each fiber possibly has both characters, and that it was difficult to divide between Aδ- and C-fibers functionally, except CV values, in the rat.[5] Thus, it seems reasonable that C-fibers respond to not only bladder distention but also bladder contractions potentially in the present study. Taken together, the present study demonstrated that mechanosensitive afferent activities of both Aδ- and C-fibers of the rat urinary bladder

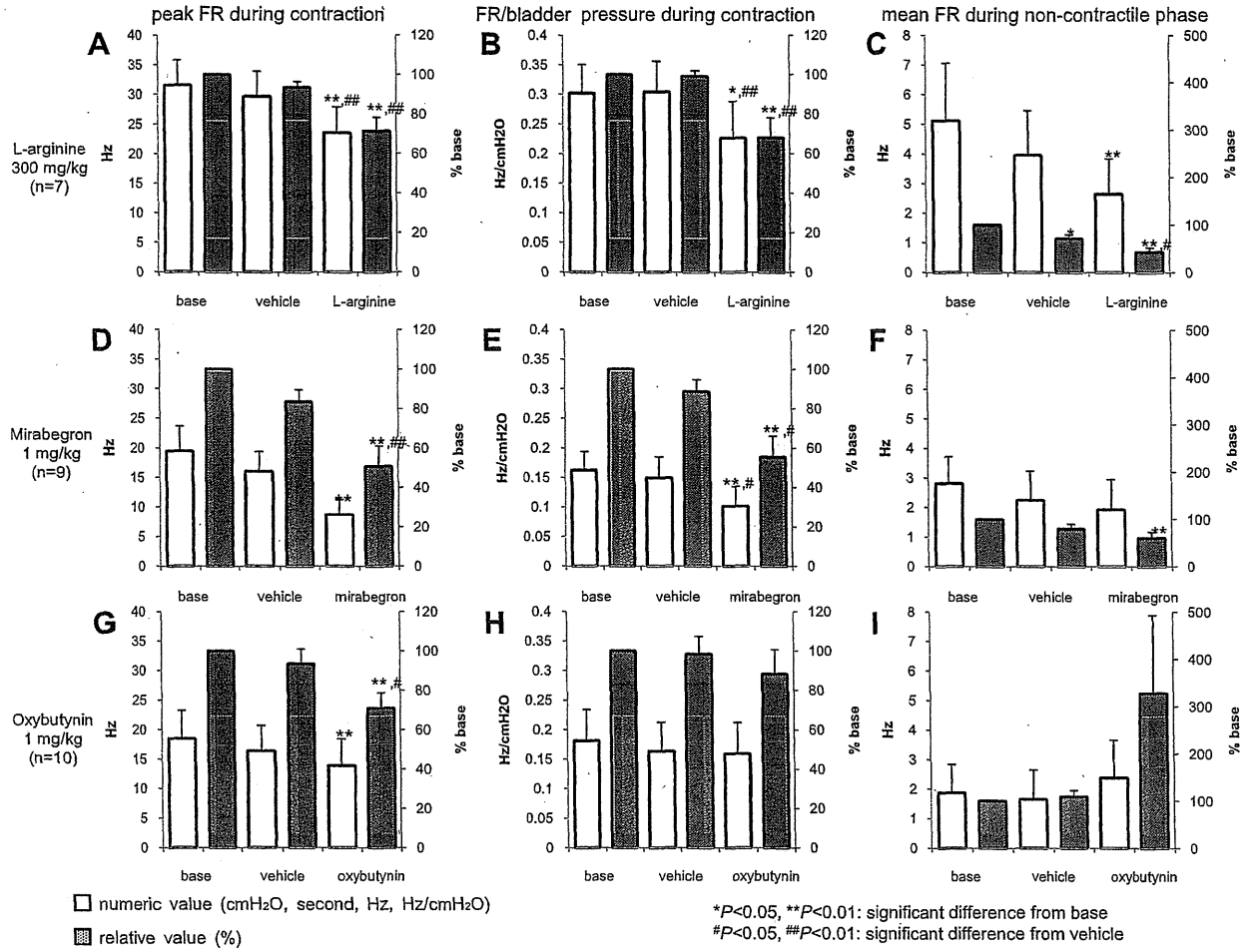

Fig. 3. Responses of afferent activities of Aδ-fibers to vehicle and drug-administrations. **A–C:** L-arginine-administration. **D–F:** Mirabegron-administration. **G–I:** Oxybutynin-administration. White bars indicate the numeric-values (correspond to left Y-axis), and gray bars indicate the relative % values (correspond to right Y-axis).

are capable of being responsive to both stretch and contractile stimuli.

We have investigated the effects of three drugs on RBCs and SAAs in this situation. After L-arginine-administration, the amplitude and duration of RBCs did not change whereas RBCs disappeared for a while as reflected the elongated TFR and TFP, and the elongated interval of RBCs. These results suggest that L-arginine can inhibit the afferent pathway from the bladder, but not the efferent pathway. This observation is consistent with the previous finding that intraurethral administration of NO donor (S-Nitroso-N-acetyl-DL-penicillamine and nitroprusside) significantly decreased the bladder contraction frequency without any changes of bladder contraction amplitude.[11] The finding that the peak of FR during RBCs after L-arginine-administration significantly decreased without any changes of bladder pressure itself may support L-arginine's selective action on the afferent pathway. Moreover, the mean FR during non-contractile phase, which may reflect the SAAs during bladder distention, was decreased after L-arginine-administrations. Our previous study during bladder filling is further supporting these findings in the present study.[9]

On the other hand, after mirabegron- and oxybutynin-administrations, the amplitude and duration of RBCs significantly decreased. In addition, the interval of RBCs elongated and the basal pressure decreased significantly, respectively, only after mirabegron-administration but not oxybutynin-administration. These results suggest that mirabegron can inhibit the both afferent and efferent pathways from/to the bladder, whereas oxybutynin inhibit only efferent pathway. Takasu et al.[12] demonstrated that mirabegron (1 mg/kg intravenously) did not significantly alter either the amplitude or frequency of RBCs, which seems partly different from our findings. In the present study, RBCs under an isovolumetric bladder condition were relatively weak and difficult to maintain continuously as described before. Such differences in experimental condition might have affected to the results of mirabegron's effect. Uchida et al. reported that intravenous oxybutynin administration larger than 0.1 mg/kg significantly reduced the amplitude but did not change frequency of RBCs in an anesthetized rat,[13] which was consistent with the present study. After mirabegron-administration, the peak of FR of SAAs during RBCs significantly decreased in Aδ-fibers, but not in C-fibers. In contrast, the peak of FR of both fiber-SAAs significantly decreased after oxybutynin-administration, but the change was not significant when the value was normalized by the amplitude of RBCs as shown in the result of FR/bladder

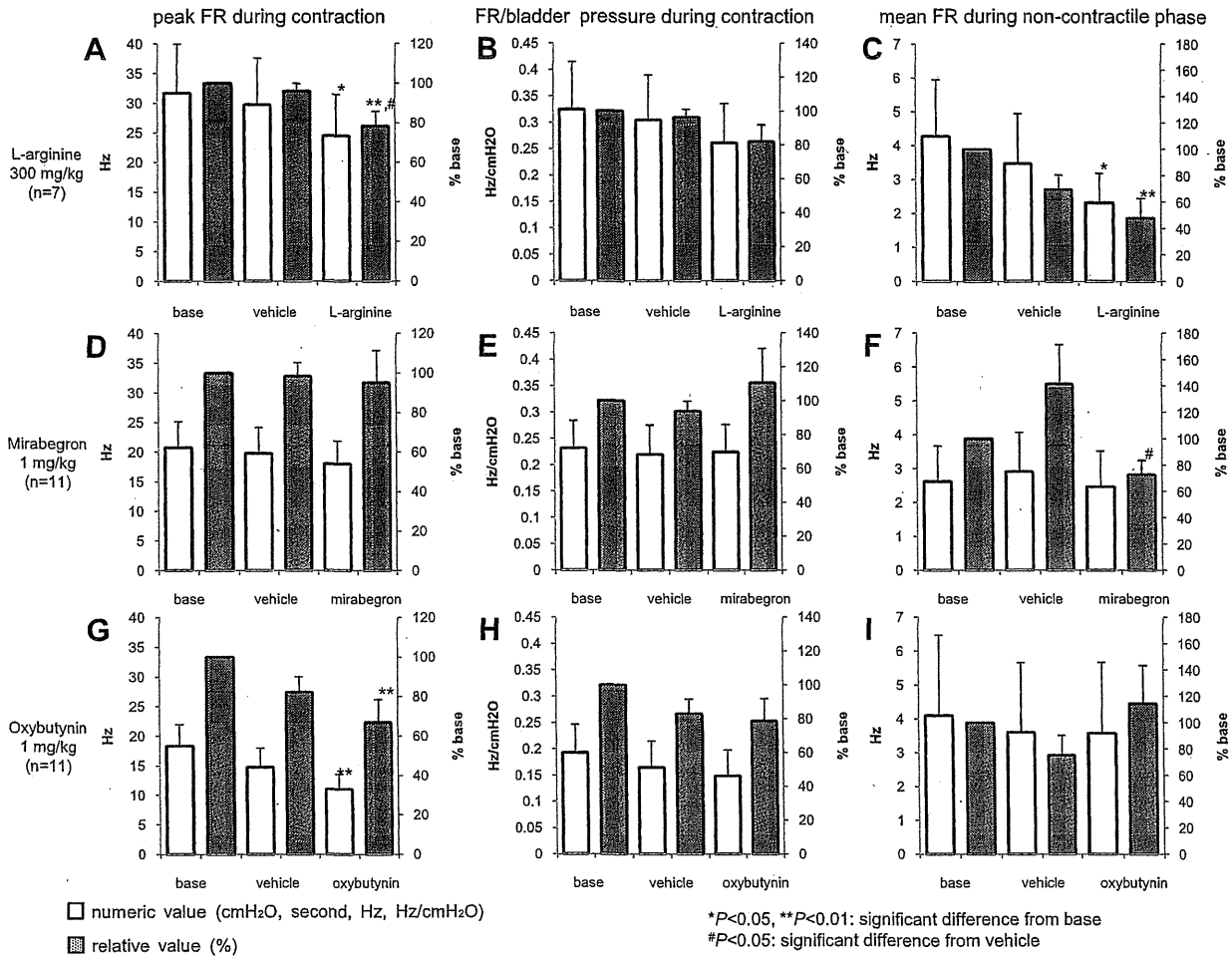

Fig. 4. Responses of afferent activities of C-fibers to vehicle- and drug-administrations. **A–C**: L-arginine-administration. **D–F**: Mirabegron-administration. **G–I**: Oxybutynin-administration. White bars indicate the numeric-values (correspond to left Y-axis), and gray bars indicate the relative % values (correspond to right Y-axis).

pressure. These results indicate that the decreased peak FR during RBCs after oxybutynin-administration might result from the weakened the bladder contractions. Taken together, these results suggest that the stimulation of β3-AR by mirabegron can inhibit mechanosensitive primary Aδ afferent activities synchronized with RBCs, but these effects were not observed with oxybutynin. Such observations with mirabegron-treatment are consistent with our previous findings on inhibition of mechanosensitive primary Aδ-afferent activities during the bladder contractions (microcontractions) of myogenic origin.[7]

It is conceivable that the activation of bladder afferent nerve fibers during RBCs demonstrated in the current study can facilitate bladder contractions during the voiding phase by providing a sensory input that is used as positive feedback to maintain the contraction of the bladder. If so, drugs that can enhance such afferent input to the CNS may be good candidates for the treatment of detrusor underactivity.

CONCLUSIONS

The present results indicate that mechanosensitive afferent activities of both Aδ- and C-fibers of the rat urinary bladder are capable of being responsive to both stretch and contractile stimuli. L-arginine, an NO substrate, inhibit the activation of both Aδ- and C-fibers (mainly Aδ-fibers) induced by bladder contractions, whereas mirabegron, a β3-AR agonist, has an inhibitory effect only on Aδ-fibers. On the other hand, oxybutynin, an antimuscarinic agent, may not have such effects. To our knowledge, this study is the first direct demonstration of the inhibitory actions of L-arginine and mirabegron on SAAs during RBCs.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Determinants of Nocturia: Pathophysiology and Assessment

Of all lower urinary tract symptoms (LUTS), nocturia is one of the most bothersome complaints of elderly men.[1] Pathophysiology of nocturia conceptually consists of the 3 elements of overproduction of nocturnal urine, small bladder capacity and sleep disorder.[1,2] These elements are likely to influence or aggravate each other, forming a complicated clinical feature of nocturia.

In this issue of The Journal van Doorn et al (page 1034) shed further light on nocturia, using frequency-volume chart variables obtained from 1,142 Dutch men 50 to 78 years old under longitudinal observation lasting 6 years.[3] The independent factors identified relative to nocturia were older age, small maximum bladder capacity (less than 300 ml), worse LUTS assessed by I-PSS (International Prostate Symptom Score) exclusive of nocturia score, 24-hour polyuria (greater than 2,800 ml/24 hours) and nocturnal polyuria (proportion of nocturnal voided volume greater than 33% of 24-hour urine volume or nocturnal urine production greater than 90 ml per hour). Comorbidities putative of risk factors such as diabetes and hypertension did not remain as independent factors. Although there are inherent limitations of an observational study, some risk factors for nocturia were identified, which offer speculative pathophysiology of nocturia in men. The authors are to be congratulated for the confirmatory findings of nocturia associated factors in their series of Krimpen longitudinal studies.[4] Sleep disorder, which was not discussed in this article, is to be investigated in a future study by the authors.

Association of nocturia with I-PSS excluding nocturia score raises the question of what other symptom score is more or less associated with nocturia. Presumably, storage symptoms such as increased micturition frequency, urgency and urgency incontinence are more related to nocturia. However, to determine this relationship, further evaluation using a more detailed and comprehensive symptom assessment is needed. The I-PSS is a questionnaire specific to symptoms of benign prostatic hyperplasia.[5] Incontinence and pain not addressed by the I-PSS could be related to nocturia,[6] most probably in those without nocturnal polyuria. In addition, a longitudinal analysis of the current data set would provide a sequential course of symptom profile in men with nocturia as to what symptom predicts it or what symptom is preceded by it.

In the study by van Doorn et al nocturnal polyuria was expressed in proportion of voided volume at night and nocturnal urine production. Both factors were calculated at bedtime and upon rising in the morning for relative nocturnal polyuria and from 1 to 6 a.m. for absolute nocturnal polyuria under the assumption that urine production is constant between 2 consecutive voids. It is intriguing that these 2 indicators, although intuitively highly correlated, were also independent significant determinants for nocturia. Absolute amount of urine rather than relative ratio of urine excreted at night was more discriminative, since an increase in relative ratio (greater than 33%) was highly prevalent in the elderly population regardless of the presence or absence of nocturia. It is probable that the relative ratio increase occurs earlier and the absolute increase occurs later. The current study could have provided insight into this assumption by sequential analysis and input from vascular and renal physiology.[7] Alternatively, a higher threshold (ie greater than 50%) may improve the discriminant ability of relative ratio.

Physiologically, the cutoffs for nocturnal factors used by van Doorn et al may not be appropriate. It is regrettable that these factors except relative amount were not adjusted for body size, ie body weight, body height, body mass index or body surface area. Body size is far from constant among races and individuals. The body size of Dutch or European men is not comparable to that of Japanese or Asian men. It is also highly variable within each country and race. Body size and body weight are fundamental clinical and physiological determinants, and the amount of urine excreted or maximum bladder capacity depends on them. Applying these cutoffs to any population or individuals studied may be difficult, although the authors indicate no specific intent of clinical implementation of their results.

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Clinical criteria or definition of nocturnal polyuria is debatable. The International Continence Society report states "nocturnal polyuria is present when greater than 20% (young adults) to 33% (over 65 years) (urine) is produced at night."[8] This definition is ambiguous in terms of age discrimination, and yet it provides the advantage of no need for body size adjustment and is probably most often used as a tentative gold standard. However, in the present study nocturnal urine production of greater than 90 ml per hour is a better alternative than the more discriminative value of greater than 33% and more evidence based.[9]

There are numerous definitions of nocturnal polyuria,[1,2] some of which are based on body size adjustment, for example 10 ml/kg body weight per night.[10] Clinical adaptation of a specific cutoff value needs scientific plausibility and generalizability. Also required is practicality, that is, measurement and calculation of urinary volume specifically for nocturnal polyuria should be simple. Pathophysiology of nocturia is complicated by multiple determinants. A common assessment scale for each potential determinant would benefit physicians and patients.

Yukio Homma
Department of Urology
Graduate School of Medicine
The University of Tokyo
Hongo, Bunkyoku, Japan

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Neurourology and Urodynamics

Influence of Urethane-Anesthesia on the Effect of Resiniferatoxin Treatment on Bladder Function in Rats with Spinal Cord Injury

Naoki Aizawa,[1] Shinichi Ogawa,[1,2] Rino Sugiyama,[1,3] Yukio Homma,[3] and Yasuhiko Igawa[1]*
[1]Department of Continence Medicine, The University of Tokyo Graduate School of Medicine, Tokyo, Japan
[2]Department of Discovery Research, Mochida Pharmaceutical Co., LTD., Shizuoka, Japan
[3]Department of Urology, The University of Tokyo Graduate School of Medicine, Tokyo, Japan

Aims: We investigated the effect of resiniferatoxin (RTX)-treatment on cystometric parameters in the spinal cord injury (SCI) rats in both conscious and urethane-anesthetized conditions and evaluated the influence of urethane-anesthesia on the effect of RTX on lower urinary tract (LUT) function in SCI rats. **Methods:** Female Sprague-Dawley rats were used. SCI was created by transection of the T8-T9 spinal cord. Four weeks after the transection, the animals were placed in a restraint cage for the first cystometric measurements in a conscious state. Secondary cystometric measurements were performed in a conscious condition following the 1 day after RTX-(0.3 mg/kg) or vehicle-subcutaneous injection. Then the animals were injected with urethane (1.5 g/kg, subcutaneously), and cystometric measurements were repeated four times every 1 hr-interval. **Results:** After the RTX-treatment in a conscious condition, urinary retention was observed in three out of five animals. In addition, the number of non-voiding contractions (NVCs) significantly decreased although their amplitude did not change significantly. After the urethane-injection, all of the animals treated with RTX developed urinary retention. The amplitude of NVCs significantly decreased, whereas the number of NVCs did not change significantly in the RTX-treated group. No cystometric parameters significantly changed after either vehicle- or urethane-injection in the vehicle-treated group. **Conclusions:** The present results indicate that the suppressive effects of RTX on NVCs as well as voiding contractions in SCI rats can be enhanced by urethane-anesthesia. Such suppressive effect of urethane-anesthesia itself should be taken into consideration when we evaluate a drug-effect on LUT function in rats with SCI. *Neurourol. Urodynam.* © 2013 Wiley Periodicals, Inc.

Key words: anesthesia; resiniferatoxin; spinal cord injury; Sprague-Dawley rats; urinary bladder

INTRODUCTION

Spinal cord injury (SCI) eliminates voluntary control of micturition and produces an initial period of detrusor areflexia, complete urinary retention that are related to the spinal shock phase. However, micturition reflex reappears at various intervals after injury and mediates automatic micturition and neurogenic detrusor overactivity (DO).[1] It has been reported that transient receptor vanilloid subfamily-1 (TRPV1) is overexpressed in the human urinary bladder following SCI.[2] For the treatment of lower urinary tract (LUT) dysfunctions induced by SCI, intravesical capsaicin (CAP), or resiniferatoxin (RTX) has been explored to produce clinical benefits for increasing bladder capacity and for decreasing DO as well as voiding bladder contractions. These clinical benefits are presumed to be attributable to desensitization of TRPV1 ion channel.[3–8] Some animal studies[9,10] demonstrated that systemic treatment with CAP inhibited only the non-voiding contractions (NVCs), of which possibly reflect as DO. In contrast to the clinical observations, these animal studies showed that the voiding bladder contractions were kept in SCI rats under conscious or a low dose of urethane-anesthetized conditions.

Urethane is the most suitable anesthetic for acute and chronic physiological experiments that require demonstration of the micturition reflex.[11] On the other hand, urethane potentiated the functions of neuronal nicotinic acetylcholine, γ-aminobutyric acid $(GABA)_A$, and glycine receptors, and it inhibited *N*-methyl-d-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in a concentration-dependent manner.[12,13] Indeed, it has been reported that the urethane-anesthesia with an usual dose (1.2 g/kg) necessary to anesthetize normal rats markedly suppressed the micturition reflex induced by bladder distension in SCI rats.[10] Thus, it is possible that the urethane can modify the effect of drugs including CAP and RTX in SCI rats. Nevertheless, most of the studies for investigating the effect of drugs on LUT function in rats following SCI have been carried out with urethane-anesthesia.[14–18]

The aims of this study were to investigate the effect of RTX-treatment on cystometric parameters in both conscious and urethane-anesthetized conditions in the same SCI rats, and to evaluate the influence of urethane-anesthesia on the effect of RTX in SCI rats.

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*Correspondence to: Yasuhiko Igawa, M.D., Ph.D., Department of Continence Medicine, The University of Tokyo Graduate School of Medicine, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: yigawa-jua@umin.ac.jp
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MATERIALS AND METHODS

Animals

Seventeen adult female Sprague-Dawley rats weighing 190–210 g (9–10 weeks-old) were used. The rats were maintained under standard laboratory conditions with a 12:12 hr light:dark cycle, and free access to food pellets and tap water. The protocol was approved by the Animal Ethics Committee of The University of Tokyo Graduate School of Medicine and in line with NIH guidelines for the care and use of experimental animals.

Surgical Procedure for SCI

Spinal transections were performed under isoflurane anesthesia at the level of T8-T9. After the laminectomy, the spinal cord was transected completely using a fine scissors. Gelfoam (Pfizer Japan, Inc., Tokyo, Japan) was placed between the severed ends of the spinal cord. The overlying muscle and skin were closed layer by layer. Each rat was housed in a separate cage. The animals were treated with an antibiotic [Gentamicin sulfate, 0.1 mg/0.1 ml/body, subcutaneously (s.c.)] for 7 days. To prevent over-distension of the bladder, urine was expressed manually every 12 hr until the time when the micturition reflex has been recovered (6–12 days after transection). Four weeks after the transection, following experiments were performed.

Experimental Procedure

A polyethylene catheter (Clay-Adams PE-50, Parsippany, NJ) was implanted into the bladder through the dome for cystometry (CMG) under anesthesia with pentobarbital sodium (35–40 mg/kg, intraperitoneally) (Fig. 1). One day after the catheter-implantation, animals were placed individually into transparent observation chamber. After 10 min adaptation period, base-line (first) eye-wipe test was performed by applying CAP solution (0.05 mg/ml, 20 μl/eye) on the eye-surface and then counting the number of eye wipes for 1.5 min, which referred to a previous study.[10] The tested eye was rinsed with 0.9% saline and swabbed after CAP application. Then, under isoflurane anesthesia, animals were placed in a restraint cage (Ballman Cage KN-326, Natsume, Tokyo, Japan) and allowed to recover from anesthesia for CMG measurements. The intravesical catheter was connected via a three-way stopcock to a pressure transducers (Nihon Kohden, Tokyo, Japan) and connected to a syringe pump (KDS 200, Muromachi Kikai Co. Ltd., Tokyo, Japan) for saline instillation at a rate of 12 ml/h. Bladder pressure was measured using data acquisition software (Power Lab, AD Instruments, Sydney, Australia) at a sampling rate of 40 Hz. CMG was repeated four times and the fourth measurement served as the Base1 value in a conscious condition. On the next day, vehicle or RTX (0.3 mg/kg) was injected s. c. under isoflurane anesthesia. One day after the vehicle or RTX injection, secondary eye-wipe test and CMG measurements as the Base2 value in a conscious condition were performed in the same way. After Base2 CMG measurement, the animals were injected with urethane (1.5 g/kg, s. c.), and CMG measurements were repeated four times every 1 hr-interval after the urethane-injection. In the CMG measurements, general cystometric parameters were analyzed. NVCs were determined as the bladder contractions whose amplitude was more than 3 cm H_2O observed for a 3-min period before micturition. In each CMG measurement, the saline-instillation for bladder filling was terminated for 40 min (at 8 ml) or when the leakage of saline through the urethra has been observed.

Drugs

Gentamicin sulfate was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). CAP and RTX were purchased from Sigma–Aldrich (St. Louis, MO). Gentamycin was dissolved in saline. CAP and RTX were dissolved in absolute ethanol as a stock solution (0.5 and 3 mg/ml, respectively) and stored at −80°C, and then subsequent dilutions of the drugs were made on the day of the experiment using saline.

Statistical Analysis

All data are expressed as mean ± SEM. Results for two group comparisons were analyzed using paired Student's *t*-test. Results for multiple comparisons were analyzed using one-way ANOVA followed by Dunnett's test. *P*-values <0.05 are considered statistically significant.

RESULTS

General Characteristics

In the RTX treated group, three animals failed to show sufficient desensitization judged by the eye-wipe test were

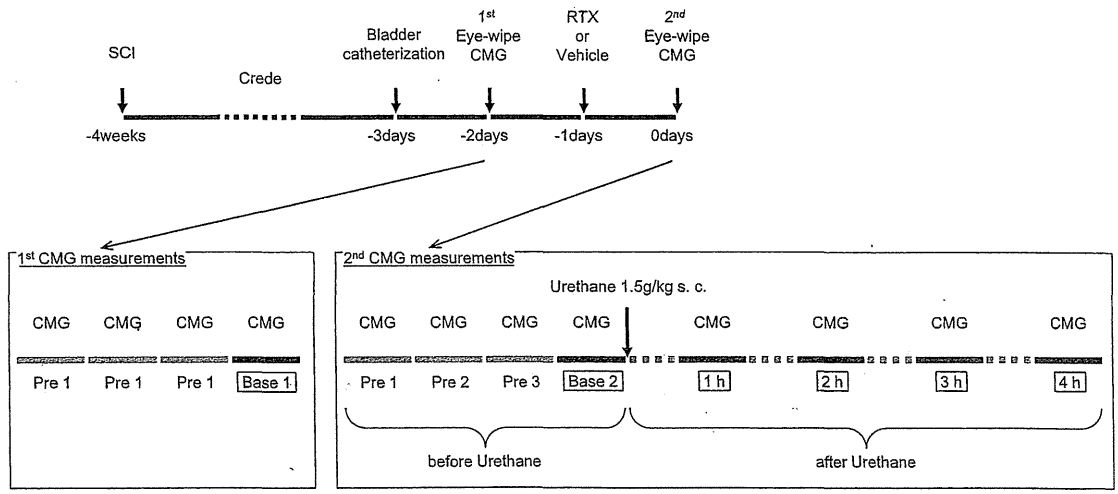

Fig. 1. Experimental procedures.

excluded for further investigations. The animals that had too small bladder capacity (<0.6 ml) or no NVCs on the base-line CMG (Base1) were also excluded (N = 4). Finally, 10 SCI animals were used for further investigations (N = 5 in each group, bladder weights of vehicle- and RTX-treated rats: 417 ± 37 and 511 ± 28 mg, respectively, $P > 0.05$).

Eye-Wipe Test and CMG Measurements

In the eye-wipe test, RTX significantly decreased the number of eye wipes, whereas those changes were not observed in vehicle-treated group (Fig. 2A).

After the RTX-treatment in a conscious condition, urinary retention was observed in three out of five animals; the voided volume significantly decreased and the residual volume and the bladder capacity increased (Fig. 2G–I). In addition, the number of NVCs significantly decreased although their amplitude did not change significantly (Fig. 2B,C). On the other hand, no parameters changed significantly after vehicle-treatment (Figs. 2 and 5).

After the urethane-injection, all of the animals treated with RTX developed urinary retention. The amplitude of NVCs significantly decreased, whereas the number of NVCs and other CMG parameters did not change significantly in the RTX-treated group (Figs. 3–5). On the other hand in the vehicle-treated group, no CMG parameters significantly changed after the urethane-injection, with an exception for a single rat developing urinary retention (Figs. 3–5).

DISCUSSION

In the present study, RTX treatment significantly decreased the behavior of eye wipes compared with before-treatment (Base1), whereas such changes were not observed in vehicle-treated group. These results are consisting with the previous findings,[10,19] and suggest that RTX can cause the desensitization of TRPV1 ion channel.[20] On the other hand, three animals failed to make this desensitization sufficiently. We used the dose of 0.3 mg/kg and the period of one day to make desensitization by RTX. This experimental procedure referred

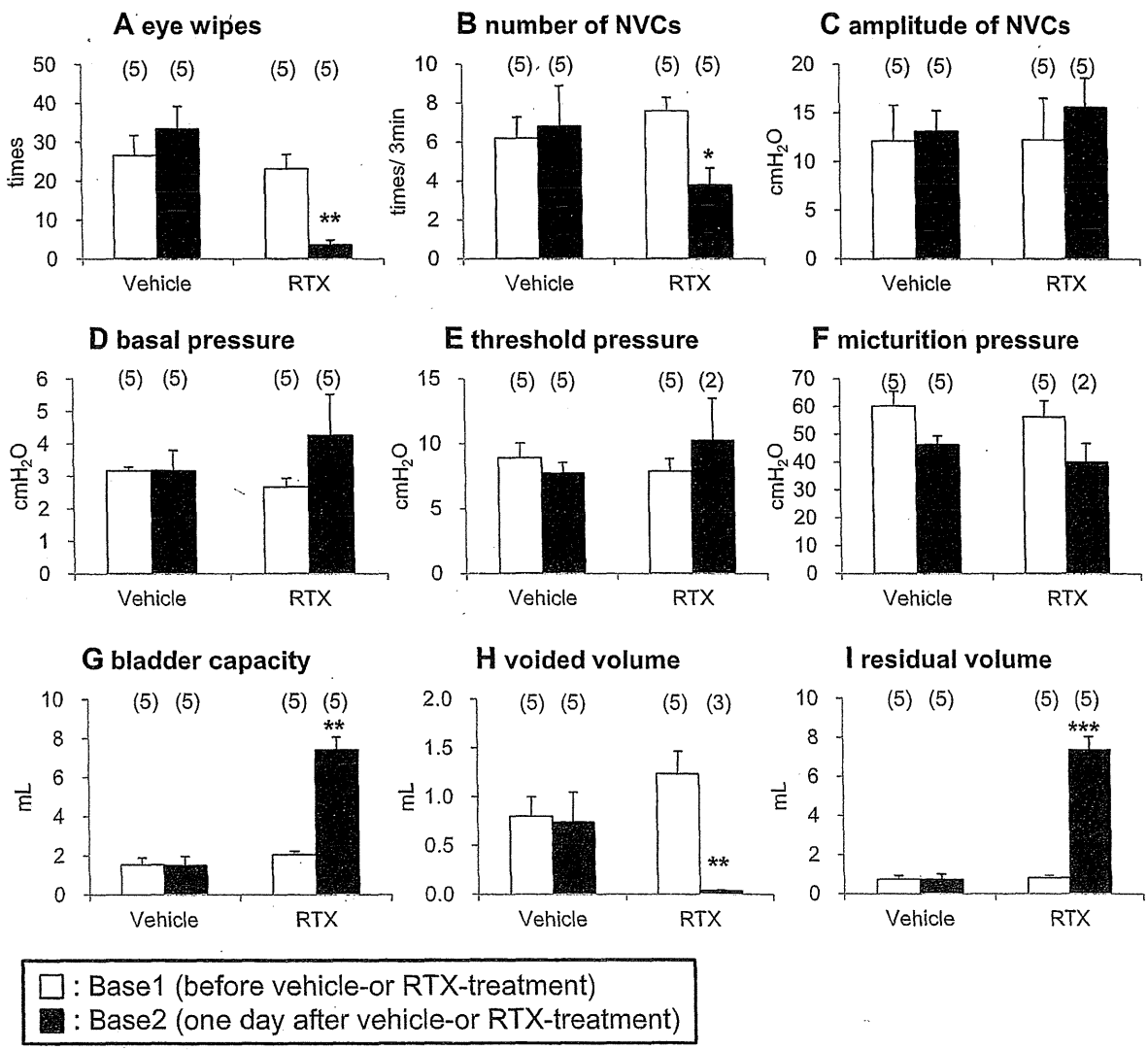

Fig. 2. Results of the eye-wipe test induced by CAP before and after vehicle or RTX treatment (A), and the effects of vehicle or RTX on cystometric parameters in conscious SCI rats (B–I). The values are expressed as mean ± SEM. The values in parenthesis show the number of rats used. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$: significant differences from Base1 (paired Student's t-test).

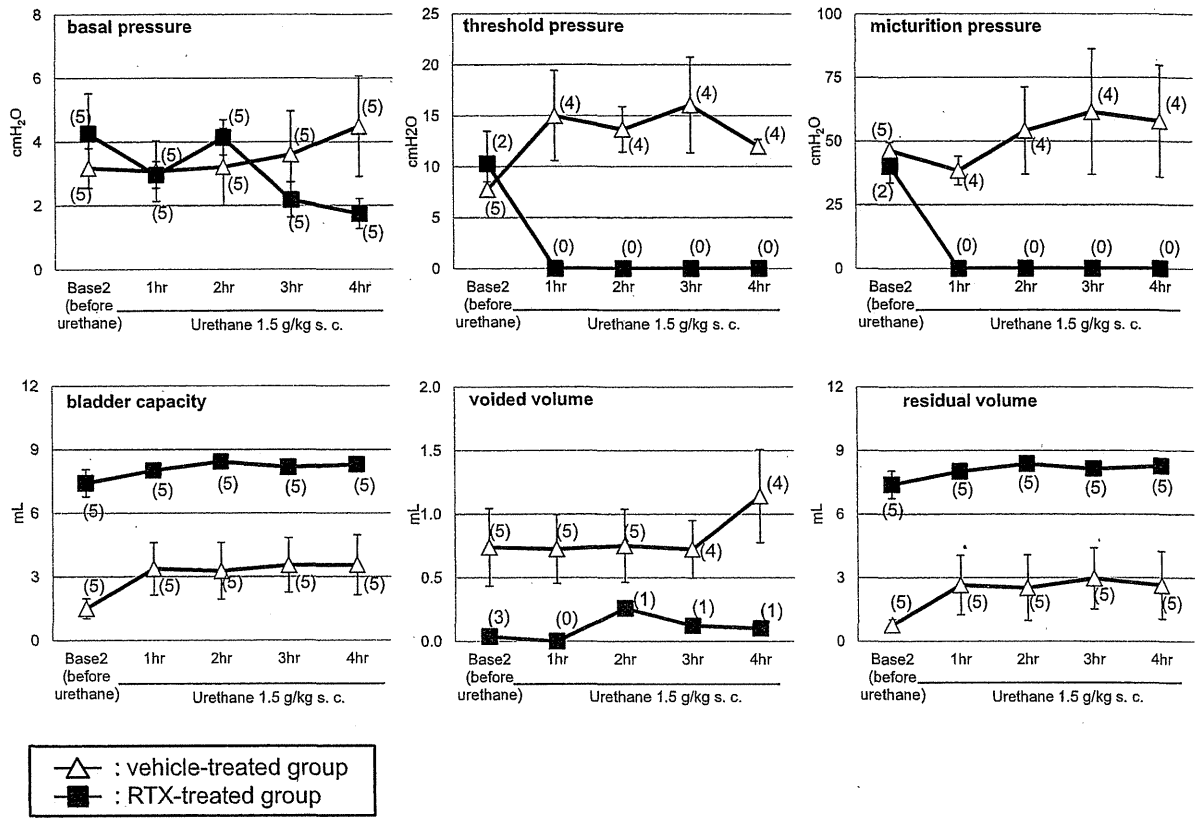

Fig. 3. The effects of before and 1–4 hr after urethane-anesthesia on cystometric parameters in vehicle- or RTX-treated SCI rats. The values are expressed as mean ± SEM. The values in parenthesis show the number of rats used. No significant differences were found in all parameters before and after urethane-anesthesia.

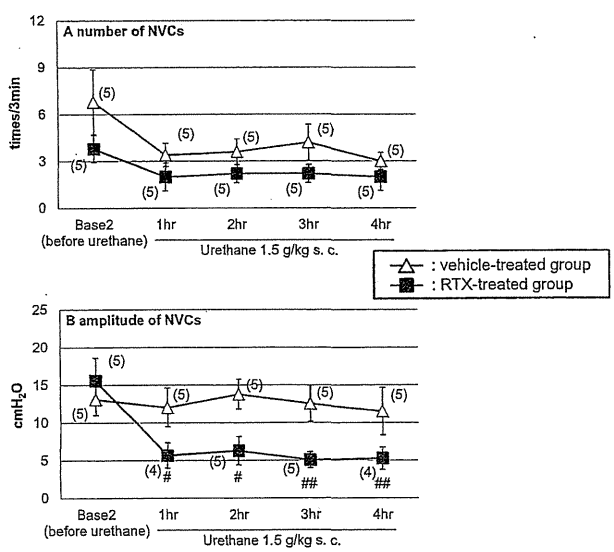

Fig. 4. The effects of before and 1–4 hr after urethane-anesthesia on amplitude (**A**) and number (**B**) of NVCs in vehicle- or RTX-treated SCI rats. The values are expressed as mean ± SEM. The values in parenthesis show the number of rats used. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$: significant differences from Base2 (one-way ANOVA followed by Dunnett's test).

to a previous study,[21] which demonstrated that the systemic RTX-pretreatment 24 hr before investigation effectively desensitized capsaicin-sensitive bladder afferents. We believe that this experimental procedure was sufficient to make the desensitization by RTX although a few animals failed to induction.

In such condition, conscious CMG measurements revealed that RTX treatment itself developed urinary retention in more than half of rats investigated, which accompanied with the decreased voided volume and the increased residual volume. In addition, the number of NVCs significantly decreased after RTX treatment. These results suggest that the desensitization induced by RTX produce the suppressive effect on the detrusor overactivity as well as micturition reflex, which lead to voiding, in conscious SCI condition. Cheng et al.[10] reported that the NVCs were dramatically attenuated in urethane-anesthetized SCI rat 4 days after treatment of systemic CAP-injection, whereas the voiding contractions were still existed. This previous study was partly inconsistent with our finding, and this discrepancy may be due to the different experimental set-up such as drugs and desensitization period. In the clinical studies, the intravesical CAP and RTX could depress DO as well as voiding contractions although there were controversy of the effects of CAP or RTX on the amplitude of the uninhibited detrusor contractions, in which the amplitude has been shown as the unchanged or the depressed.[3,4,22,23] Therefore, our finding of the effects of RTX treatment under a conscious condition may be in line with the clinical observations.

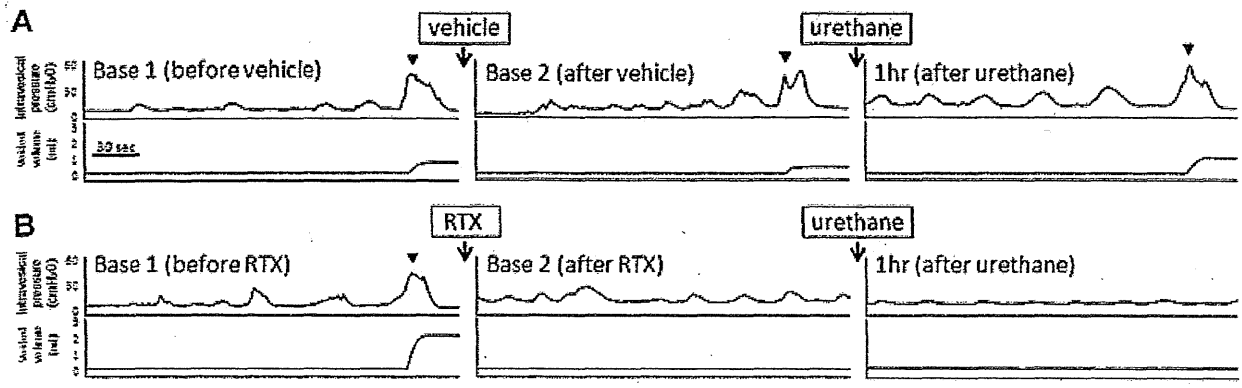

Fig. 5. Representative traces of CMG in each group. **A**: Vehicle-treated group, before and after vehicle and urethane-anesthesia. **B**: RTX-treated group, before and after RTX and urethane-anesthesia.

Cheng et al. investigated the bladder functions before and at various times after the induction of urethane-anesthesia (1.2 g/kg, s.c.) in CMG measurements. They indicated that the bladder reflex contractions and NVCs in all of the SCI animals (n = 4) were completely blocked at 3 hr after induction of anesthesia.[10] In addition, they also reported that the injection of urethane-anesthesia at less dose (0.8 g/kg, s.c.) produced the disappearance of the urine voiding in 45% (8/18) in vehicle (for CAP)-treated SCI rats, and the remainder (10/18) of the animals excreted only small amounts of fluid (10% of bladder volume eliminated) during distension of the bladder even though they exhibited large amplitude bladder contractions.[9] Thus, they concluded that urethane-anesthesia itself markedly suppress the bladder micturition reflex activities and NVCs. In the present study, we found only one animal which developed urinary retention after urethane-anesthesia in vehicle-treated rats. And, we did not find any cystometric changes, including NVCs and voiding bladder contraction parameters, with higher dose of urethane (1.5 g/kg, s.c.) than the previous studies used. The experimental set-up between these previous findings and our findings were similar, nevertheless, there were large discrepancies of the results between them, and we did not find any exact reasons for these discrepancies.

On the other hand in RTX-treated rats, 1–4 hr after urethane-anesthesia, all of the animals developed urinary retention. In addition, the amplitude of NVCs decreased although the number of NVCs did not change. Regarding the unchanged number of NVCs following the urethane-anesthesia, it is conceivable that the further reduced response could not be detected after urethane because this value has been already reduced after RTX treatment although the value tended to decrease. Since urethane-anesthesia itself did not show any cystometric changes in vehicle-treated rats, urethane-anesthesia has a potentially synergic suppressive effect on RTX induced desensitization in SCI rats.

In the clinical investigation in the patients with SCI, intravesical CAP or RTX induced the increase in bladder capacity and the decrease in DO as well as voiding detrusor contractions.[3–8] These reports are consistent with our findings in SCI rats under a conscious condition. However, the effects of desensitization are enhanced if this investigation is performed under urethane-anesthesia, and this may not reflect the clinical observations. Thus, at least in rats with SCI, pharmacological studies may need to carry out without urethane-anesthesia to evaluate drug-effects appropriately.

CONCLUSIONS

The present results indicate that the suppressive effect of RTX on non-voiding contractions as well as voiding contractions in SCI rats can be enhanced by urethane-anesthesia. Such suppressive effect of urethane-anesthesia itself should be taken into consideration when we evaluate a drug-effect on LUT function in rats with SCI.

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Perspectives in Urology

Hypersensitive bladder: Towards clear taxonomy surrounding interstitial cystitis

Yukio Homma

Department of Urology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Correspondence: Yukio Homma M.D., Department of Urology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: homma-uro@umin.ac.jp

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There is confusion surrounding the taxonomy of interstitial cystitis (IC) and its related symptom syndromes, painful bladder syndrome (PBS), bladder pain syndrome (BPS) and overactive bladder (OAB) syndrome.[1]

IC is a disease name long used in medical and consumer societies, but it lacks clear definition.[2] PBS is defined by the International Continence Society (ICS) as "suprapubic pain related to bladder filling, accompanied by other symptoms, such as increased daytime and night-time frequency, in the absence of proven urinary infection or other obvious pathology",[3] PBS[4,5] and BPS[6] definitions by other societies all share two requirements: symptoms including pain and lack of identifiable diseases explaining the symptoms. Chimeric terms combining IC and syndromes, IC/PBS (IC/BPS), apparently indicate conditions presenting the symptom complex in the absence of obvious diseases with the possible presence of IC. However, these terms are used interchangeably. In addition, PBS and BPS contain "pain", creating a misunderstanding that the patient must complain of pain, although a substantial proportion of patients do not. Complaints commonly heard are not directly linked to pain; that is, incessant need to void due to discomfort, loss of normal urinary sensation and an irritable sensation with only little urine in the bladder. These symptoms suggest increased sensation of the bladder or urothelium, a highly sensitive sensory tissue,[7] and an adequate term for such a condition is lacking.

I should therefore like to propose hypersensitive bladder (HSB)[8] (although "hypersensitive" can be replaced by other words implying increased sensation). HSB syndrome is a condition with HSB symptoms with no obvious diseases. HSB symptoms are defined as "increased bladder sensation, usually associated with urinary frequency and nocturia, with or without bladder pain". They are similar to OAB symptoms; urinary urgency, usually associated with urinary frequency and nocturia, with or without urgency incontinence.[3] Increased bladder sensation is described as "early and persistent desire to void".[3] IC is defined as a disease requiring: (i) HSB symptoms; (ii) no obvious diseases; and (iii) bladder pathology, such as Hunner's ulcer or mucosal bleeding after overdistension (Fig. 1a). Symptoms of OAB and HSB substantially overlap, whereas OAB with leakage and HSB with pain are readily separated. The compelling need to void in OAB is characterized by the sudden onset and/or fear of leakage, whereas that in HSB is of a persistent unpleasant sensation and/or fear of pain.[9] IC is a representative disease causing HSB symptoms, most typically with pain, but might be painless and indistinguishable from OAB (Fig. 1b).

With these definitions, the following make sense: (i) a woman complaining of HSB symptoms proved to have carcinoma *in situ* of the bladder; (ii) a woman with HSB symptoms was diagnosed as HSB because no obvious diseases were apparent, but now she is diagnosed with IC based on cystoscopic abnormality; and (iii) a man with HSB showed no definite findings for IC at hydrodistension, so he remained as HSB.

Introducing HSB, the counter concept of OAB, into bladder dysfunction taxonomy will clarify the current confusion surrounding IC.

Conflict of interest

None declared.

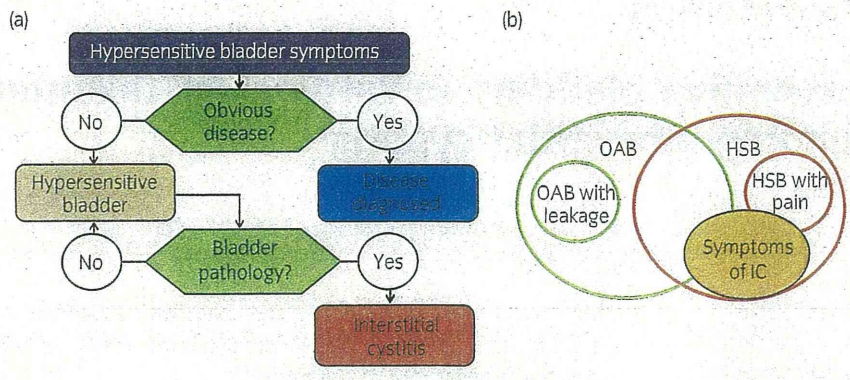

Fig. 1 Relationship of hypersensitive bladder (HSB) and its related terms. (a) Hypersensitive bladder symptoms (increased bladder sensation, usually associated with urinary frequency and nocturia, with or without bladder pain) are to be explored for the causes. When there is no obvious disease, the condition is called HSB. Furthermore, when bladder pathology (such as Hunner's ulcer or mucosal bleeding after overdistension) is identified in HSB, the diagnosis of interstitial cystitis (IC) is made. When there is no such bladder abnormality, the condition remains as HSB. (b) Symptoms of overactive bladder (OAB; urinary urgency, usually associated with urinary frequency and nocturia, with or without urgency incontinence) and HSB are substantially overlapping, although OAB with leakage and HSB with pain can be readily discriminated. Symptoms of IC mostly present as HSB symptoms (typically with pain), but occasionally can be indistinguishable from OAB.

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Increased mRNA Expression of Genes Involved in Pronociceptive Inflammatory Reactions in Bladder Tissue of Interstitial Cystitis

Yukio Homma,*,† Akira Nomiya,‡ Mitsuhiro Tagaya,§ Tatsuya Oyama,§ Kazuchika Takagaki,§ Hiroaki Nishimatsu|| and Yasuhiko Igawa¶

From the Department of Urology, University of Tokyo (YH, AN, HN) and Department of Continence Medicine, University of Tokyo Graduate School of Medicine (YI), Tokyo and Research Laboratories, Nippon Shinyaku Co., Ltd. (MT, TO, KT), Kyoto, Japan

Purpose: We assayed mRNA expression of the TRP family of channels and ASIC1 in bladder tissue from patients with interstitial cystitis.

Materials and Methods: Bladder biopsies of 1) nonclassic interstitial cystitis, 2) nonulcerative portions of classic interstitial cystitis, 3) ulcerative portions of classic interstitial cystitis and 4) noncancerous portions of bladder cancer as the control were placed immediately in ice-cold RNAlater® and subjected to real-time reverse transcriptase-polymerase chain reaction. We compared the mRNA expression of TRP channels, ASIC1, NGF, CXCL9 and UPK3A with that of controls, and correlated expression with symptom severity.

Results: We analyzed specimens from 17 patients with nonclassic interstitial cystitis, 22 with classic interstitial cystitis and 11 controls. In nonclassic interstitial cystitis samples TRPV2 and NGF showed significantly increased expression. In classic interstitial cystitis samples nonulcerative portions demonstrated a significant increase in the expression of TRPA1, TRPM2 and 8, TRPV1 and 2, ASIC1, NGF and CXCL9, and a significant decrease in UPK3A and TRPV4. Ulcerative portions showed similar changes for TRPM2, TRPV1, 2 and 4, CXCL9 and UPK3A. Increased expression of TRPM2, first noted in interstitial cystitis tissue, was the most pronounced one of the TRP family. All symptom measures correlated with TRPM2 and TRPV2 expression, and partially with that of the other genes.

Conclusions: This study showed increased expression of the genes involved in pronociceptive inflammatory reactions in interstitial cystitis, including TRPV1, 2 and 4, ASIC1, NGF and CXCL9, and to our knowledge TRPM2 for the first time. The different expression patterns suggest distinct pathophysiologies for classic and nonclassic interstitial cystitis. The genes and their products are potential candidates for use as biomarkers or novel therapy targets.

Abbreviations and Acronyms

ASIC = acid sensing ion channel
CXCL9 = chemokine (C-X-C motif) ligand 9
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
IC = interstitial cystitis
NGF = nerve growth factor
OSPI = O'Leary-Sant problem score
OSSI = O'Leary-Sant symptom score
qRT-PCR = quantitative real-time RT-polymerase chain reaction
RT = reverse transcriptase
TRP = transient receptor potential
TRPA = TRP ankyrin
TRPM = TRP melastatin
TRPV = TRP vanilloid
UPK = uroplakin
VAS = visual analog pain scale

Key Words: urinary bladder; cystitis, interstitial; gene expression; transient receptor potential channels; uroplakins

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* Correspondence: Department of Urology, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan (telephone: +81 3 5800 8911; FAX: +81 3 5800 6435; e-mail: homma-uro@umin.ac.jp).
† Financial interest and/or other relationship with Astellas, Taiho, Pfizer, Ono, Daiichisankyo, Asahi Kasei and GlaxoSmithKline.
‡ Financial interest and/or other relationship with Taiho Pharmaceutical, Pfizer, Astellas Pharma and Asahi Kasei Pharma.
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