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Effects of Mirabegron, a Novel β3-Adrenoceptor Agonist, on Primary Bladder Afferent Activity and Bladder Microcontractions in Rats Compared With the Effects of Oxybutynin

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Abstract

Background: Mirabegron is the first β3-adrenoceptor agonist that is clinically effective for overactive bladder.
Objective: The effects of mirabegron on primary bladder mechanosensitive single-unit afferent activities (SAAs) and bladder microcontractions were evaluated and compared with the effects of oxybutynin.
Design, setting, and participants: Female Sprague-Dawley rats were anesthetized. The SAAs generated from left L6 dorsal roots were identified by electrical stimulation of the left pelvic nerve and bladder distension. Nerves with conduction velocities (CVs) >2.5 m/s were designated as Aδ-fibers, and nerves with CVs < 2.5 m/s were designated as C-fibers.
Outcome measurements and statistical analysis: Two measurements were performed in separate animals. First, after measuring the baselines of SAA during constant filling cystometry, the procedure was repeated with each intravenous administration of mirabegron at three doses—0.1, 0.3, and 1.0 mg/kg—cumulatively. Second, the bladder was filled with saline until the intravesical pressure reached 30 cm H_2O and was kept under an isovolumetric condition; then the recording was performed for 5 min with vehicle and mirabegron or oxybutynin administrated intravenously.
Results and limitations: A total of 74 single-unit afferent fibers were isolated from 55 rats (Aδ-fibers: $n = 34$; C-fibers: $n = 40$). SAAs of both Aδ-fibers and C-fibers in response to bladder filling significantly decreased after mirabegron administration in a dose-dependent manner, which was more remarkable for Aδ-fibers. During an isovolumetric condition of the bladder, the mean bladder pressure and the number of microcontractions decreased after mirabegron administration, whereas these parameters did not change with oxybutynin administration. SAAs of Aδ-fibers were significantly decreased by mirabegron administration at both 0.3 and 1 mg/kg, whereas SAAs of C-fibers decreased only at 1 mg/kg. In contrast, oxybutynin (1 mg/kg) did not alter either type of SAA.
Conclusions: The present study demonstrates that mirabegron can inhibit mechanosensitive bladder afferent activity, especially of Aδ-fibers, which may be related to suppression of bladder microcontractions.

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1. Introduction

In the treatment of overactive bladder (OAB), anticholinergic (antimuscarinic) agents such as oxybutynin, darifenacin, and tolterodine have been widely used. However, these agents have various disadvantages, such as causing dry mouth and constipation, and they have the potential for increasing voiding difficulty in patients with bladder outlet obstruction (BOO) or bladders with poor contractility. Recent studies demonstrated that some of these drugs have an inhibitory effect on not only the efferent pathway but also primary bladder afferent activities in rats [1–3].

The β3-adrenoceptor (β3-AR) mRNA is expressed predominantly in the human detrusor compared with other β-AR subtypes (β1-AR and β2-AR), and it is suggested that β3-AR contributes to urine storage by relaxing the detrusor muscle [4–8]. β3-AR agonists are proposed as a new drug class in the treatment of OAB. Mirabegron, a β3-AR agonist, has a high selectivity for β3-AR and a higher agonist activity not only for rat bladder but also for human bladder 20–200 times greater than other agonists such as CL316,243 [9]. This drug has been shown to be clinically effective in randomized placebo-controlled phase 2 and phase 3 studies in OAB [10–12] and is approved in Japan as the first β3-AR agonist for the treatment of OAB. The activation of β3-AR increases bladder capacity, with less influence on bladder contraction or residual urine volume during the voiding phase [4] because of the pharmacologically different mechanisms of the anticholinergic agent compared with the β3-AR agonist.

It has been proposed that β3-AR agonists inhibit not only the efferent but also the afferent pathways innervating the bladder via release of nitric oxide or an unidentified inhibitory factor from the urothelium [13–16], which actively participates in sensory functions, expressing various receptors for neurotransmitters and releasing neurotransmitters in response to various stimuli [17]. A previous study demonstrated that CL316,243, a selective β3-AR agonist, can inhibit the mechanosensitive Aδ-fibers but not the C-fibers of the primary bladder afferents in rats [18]. Other previous studies demonstrated that this drug reduced bladder nonvoiding contractions [19] of myogenic origin [20] in a rat model of a partial BOO. These myogenic autonomous bladder activities may generate localized microcontractions. It has been proposed that such localized microcontractions facilitate afferent activities even in the normal human, guinea pig, and rat bladder [21–23] and may play a key role in sensory functions such as urgency.

In the present study, we investigated the effects of mirabegron, a novel β3-AR agonist, directly on single-fiber activities of the primary bladder afferent nerves and bladder microcontractions and compared these effects with those of oxybutynin, an anticholinergic agent, in the normal rat.

2. Materials and methods

2.1. Animals

Fifty-five adult female Sprague-Dawley rats weighing 180–242 g (aged 9–11 wk) were used. The rats were maintained under standard laboratory conditions with a cycle of 12 h light and 12 h dark 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 was in line with National Institutes of Health guidelines for the care and use of experimental animals.

2.2. Detection and classification of mechanosensitive bladder afferent activity

The rats were anesthetized with urethane (1.2 g/kg intraperitoneally). Body temperature was maintained by a heated blanket at 38 °C. Single afferent fiber measurements were performed as described before [18,24,25]. In brief, 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) was inserted in the bladder. Both L6 dorsal roots were cut close to their entrance to the spinal cord after the laminectomy. Fine filaments were dissected from the left L6 dorsal root and placed across shielded bipolar silver electrodes. Clearly different unitary action potentials of afferent fibers 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 impulse shape recognition program (CED, Cambridge, UK). Conduction velocity (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 anatomic data. Fibers were grouped based on CV. Fibers with a CV <2.5 m/s were considered to correspond to unmyelinated C-fibers, and fibers with CV ≥ 2.5 m/s were considered to correspond to myelinated Aδ-fibers [26]. After detecting and classifying these mechanosensitive afferent activities, two experiments were performed as follows.

2.3. Afferent measurement during constant filling of the bladder (n = 15)

Single-fiber afferent activity was recorded during constant filling cystometry with saline at 0.08 ml/min after the bladder was emptied. Filling continued until an intravesical pressure of 30 cm H_2O was reached. The afferent activity caused by pelvic nerve stimulation was also recorded before and after bladder filling and was confirmed to correspond with the afferent activity caused by bladder filling. At the beginning of the experiments, recording was repeated consecutively three times, at 5-min intervals, to evaluate the reproducibility. The third recording served as the baseline value. After measuring the baselines of afferent activity during constant filling cystometry, mirabegron at three doses (0.1, 0.3, and 1.0 mg/kg) or vehicle was intravenously administrated cumulatively. Three minutes after each dose administration of mirabegron or vehicle, consecutive bladder fillings were performed with saline.

Unitary afferent activity (firing rate) was evaluated in relation to intravesical pressure and volume. The relationship of nerve activity to pressure or volume was established by comparing nerve activity and intravesical pressure or volume at 1-s intervals. These values were then averaged at a 5-cm H_2O interval of pressure or by dividing into five equal parts of volume in the filling phase. Average unitary activity was totaled as a function of intravesical pressure or volume. Afferent nerve activity was expressed as a percentage of baseline activity and a numeric value, integrated for the whole filling phase. The numeric values of bladder compliance and the number and mean amplitude of microcontractions (based on peak to through for each microcontraction) were calculated between the start and the end of the filling phase. The cutoff value of the amplitude of microcontraction was defined as 1.5 cm H_2O, and when no microcontraction with an amplitude ≥ 1.5 cm H_2O was found, the amplitude of microcontraction was defined as 0 cm H_2O.

2.4. Afferent measurement during an isovolumetric condition of the bladder (n = 40)

The bladder was emptied and saline instilled at a rate of 0.08 ml/min until the intravesical pressure reached 30 cm H_2O. The bladder was kept under an isovolumetric condition and allowed to stabilize for 5 min, and then vehicle was administrated intravenously and the recording was performed for 5 min. The bladder pressure, microcontractions, and single afferent activities were analyzed for 3 min before and after each administration. The procedure was repeated with intravenous administration of mirabegron (0.3 or 1 mg/kg) or oxybutynin (1 mg/kg) instead of vehicle. The values were represented as percentage of baseline values and were compared between vehicle and drug administrations.

2.5. Drugs

N,N-Dimethylacetamide (DMA) and oxybutynin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cremophor was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Mirabegron, (*R*)-2-(2-aminothiazol-4-yl)-4′-[9]acetanilide, was provided from Astellas Pharma Inc. (Tokyo, Japan). Mirabegron was dissolved in 5% DMA, 5% Cremophor, and 90% distillated water. Oxybutynin was dissolved in saline. Drugs and vehicle were administrated intravenously through a polyethylene catheter (PE-50) placed in the right external jugular vein.

2.6. Statistical analysis

All data are expressed as mean ± standard error of the mean. Results for two group comparisons were analyzed using the unpaired Student *t* test. Results for multiple comparisons were analyzed using the two-way analysis of variance or Friedman test followed by the Tukey test for relative or numeric value comparisons, respectively. Values of $p < 0.05$ are considered statistically significant.

3. Results

3.1. Afferent characterization

A total of 74 single-unit afferent fibers were isolated from 55 rats. Thirty-four units corresponded to criteria for myelinated Aδ-fibers (CV: 5.07 ± 0.56 m/s), and 40 units corresponded to criteria for unmyelinated C-fibers (CV: 1.58 ± 0.06 m/s).

3.2. Effect of vehicle and mirabegron on bladder compliance and microcontractions during constant filling

No significant differences were found before and after vehicle administration in any of the parameters analyzed (Table 1). After mirabegron administration, the number of microcontractions was significantly decreased in a dose-dependent manner, whereas neither the amplitude of microcontractions nor bladder compliance changed significantly (Table 2).

3.3. Effects of mirabegron on Aδ-fiber and C-fiber activities during constant filling (n = 15)

Mechanosensitive single afferent activities of both Aδ-fibers and C-fibers were significantly decreased after mirabegron administration in a dose-dependent manner, which was more remarkable for Aδ-fibers than C-fibers. Such changes were not found after vehicle administration (Figs. 1–3).

Table 1 – Numeric values of bladder compliance (milliliters per centimeter of water), numbers and amplitude of microcontractions, and afferent activities (Aδ-fibers and C-fibers) before and after vehicle administration (5% N,N-dimethylacetamide, 5% Cremophor, and 90% distillated water)

Parameters		Baseline (before)	After–1	After–2	After–3	No.
Bladder compliance, ml/Δcm H_2O		0.0173 ± 0.0011	0.0180 ± 0.0011	0.0187 ± 0.0012	0.0190 ± 0.0012	9
Microcontractions	No.	9.33 ± 3.03	7.89 ± 2.57	8.56 ± 2.94	8.67 ± 3.14	
(>1.5 cm H_2O)	Amplitude, cm H_2O	1.66 ± 0.43	1.58 ± 0.41	1.61 ± 0.43	1.67 ± 0.44	
Aδ-fibers, Hz	Based on pressure	19.53 ± 5.30	20.63 ± 5.83	20.33 ± 4.41	22.69 ± 4.77	7
	Based on volume	15.50 ± 4.73	14.56 ± 4.06	14.53 ± 3.22	15.47 ± 3.13	
C-fibers, Hz	Based on pressure	15.89 ± 8.35	14.99 ± 7.42	15.48 ± 7.12	14.38 ± 6.52	7
	Based on volume	12.27 ± 6.87	10.14 ± 4.72	10.23 ± 4.21	9.69 ± 3.92	

The values are indicated as mean plus or minus standard error of the mean. No significant differences were found in any parameter.

Table 2 – Numeric values of bladder compliance (milliliters per centimeter of water), numbers and amplitude of microcontractions, and afferent activities (Aδ-fibers and C-fibers) before and after mirabegron administration (0.1, 0.3, and 1 mg/kg administered intravenously)

Parameters		Baseline (before)	After 0.1 mg/kg	After 0.3 mg/kg	After 1 mg/kg	No.
Bladder compliance, ml/Δcm H_2O		0.0208 ± 0.0019	0.0222 ± 0.0022	0.0224 ± 0.0025	0.0238 ± 0.0029	9
Microcontractions	No.	10.89 ± 2.82	10.78 ± 2.78	4.78 ± 1.88*	4.00 ± 1.91**	
(>1.5 cm H_2O)	Amplitude, cm H_2O	2.16 ± 0.40	2.28 ± 0.42	1.97 ± 0.56	1.55 ± 0.51	
Aδ-fibers, Hz	Based on pressure	39.50 ± 17.62	30.88 ± 10.60	23.53 ± 8.78	20.45 ± 9.21**	6
	Based on volume	21.12 ± 7.68	23.06 ± 9.13	12.97 ± 5.35	10.02 ± 4.26**	
C-fibers, Hz	Based on pressure	29.01 ± 9.86	27.78 ± 9.21	26.60 ± 9.82	20.01 ± 7.45**	7
	Based on volume	15.25 ± 4.92	14.13 ± 4.43	12.69 ± 4.51	10.03 ± 3.68**	

The values are indicated as mean plus or minus standard error of the mean.
* $p < 0.05$.
** $p < 0.01$; significant differences from baseline value (the Friedman test followed by the Tukey test).

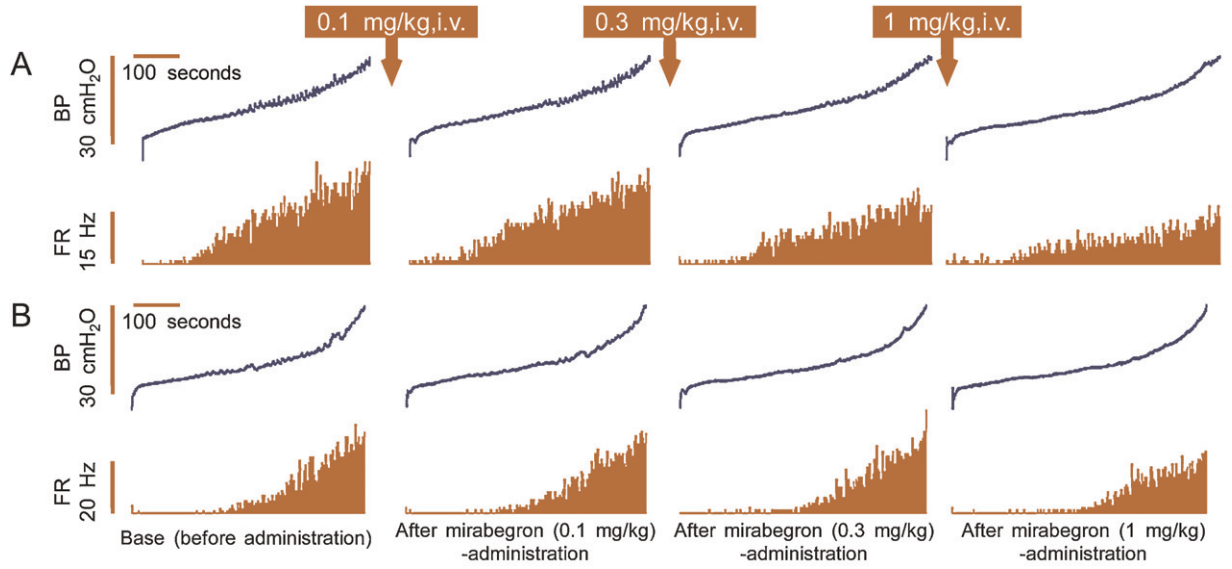

Fig. 1 – Representative recordings of bladder pressure (BP) and firing rate (FR) of (A) Aδ-fibers and (B) C-fibers during bladder filling with saline before (baseline) and after mirabegron administration. After the cumulative mirabegron administration, the FR of the Aδ-fibers and C-fibers during bladder filling decreased from baseline in a dose-dependent manner. Such a decreased response was more remarkable for Aδ-fibers than C-fibers. The inhibition of afferent activities appeared to synchronize with the decrease in fluctuations in bladder pressure. IV = intravenous.

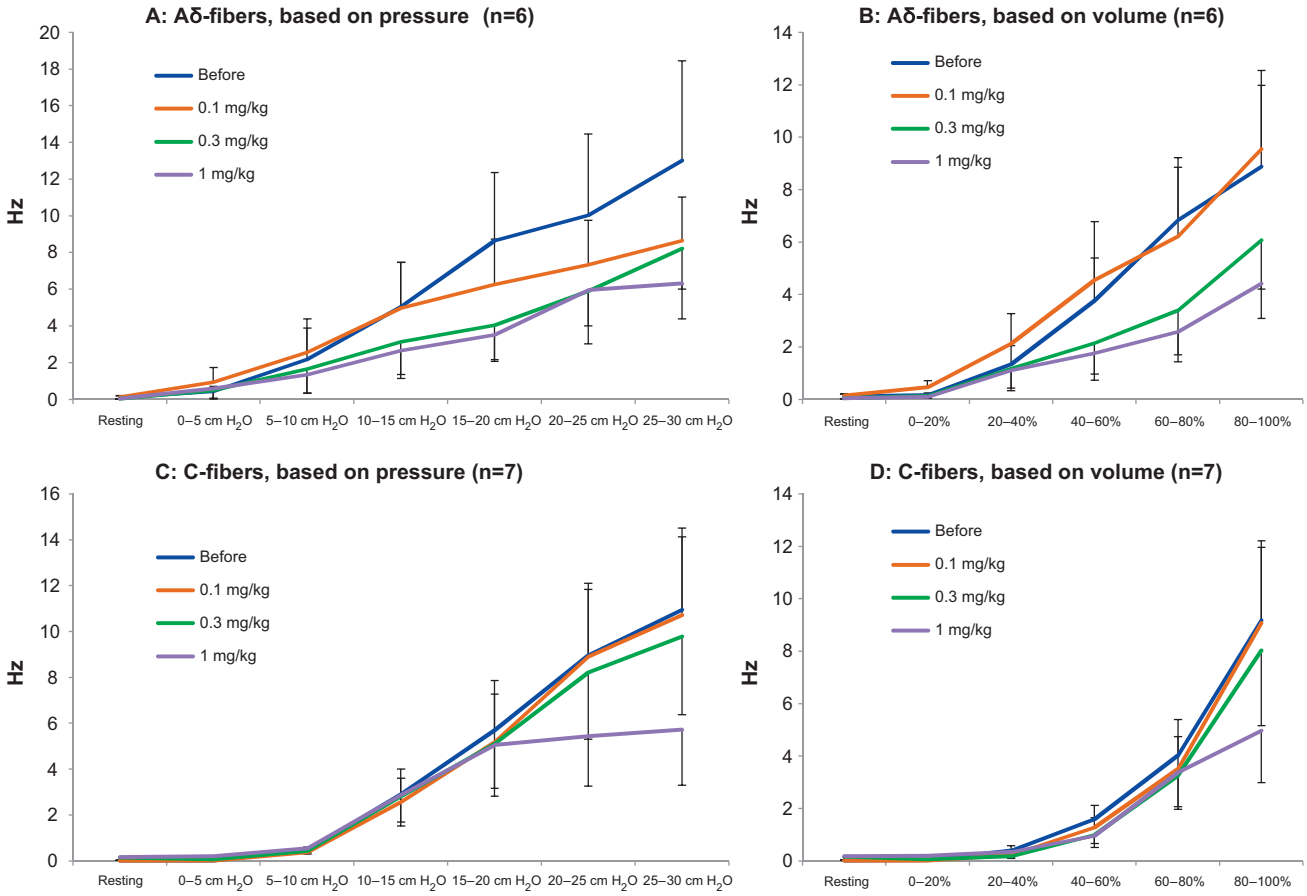

Fig. 2 – Influence of mirabegron administration (0.1–1 mg/kg) on pressure- or volume-related mechanosensitive afferent nerve activity in (A, B) Aδ-fibers and (C, D) C-fibers. Each value represents mean ± standard error of the mean.

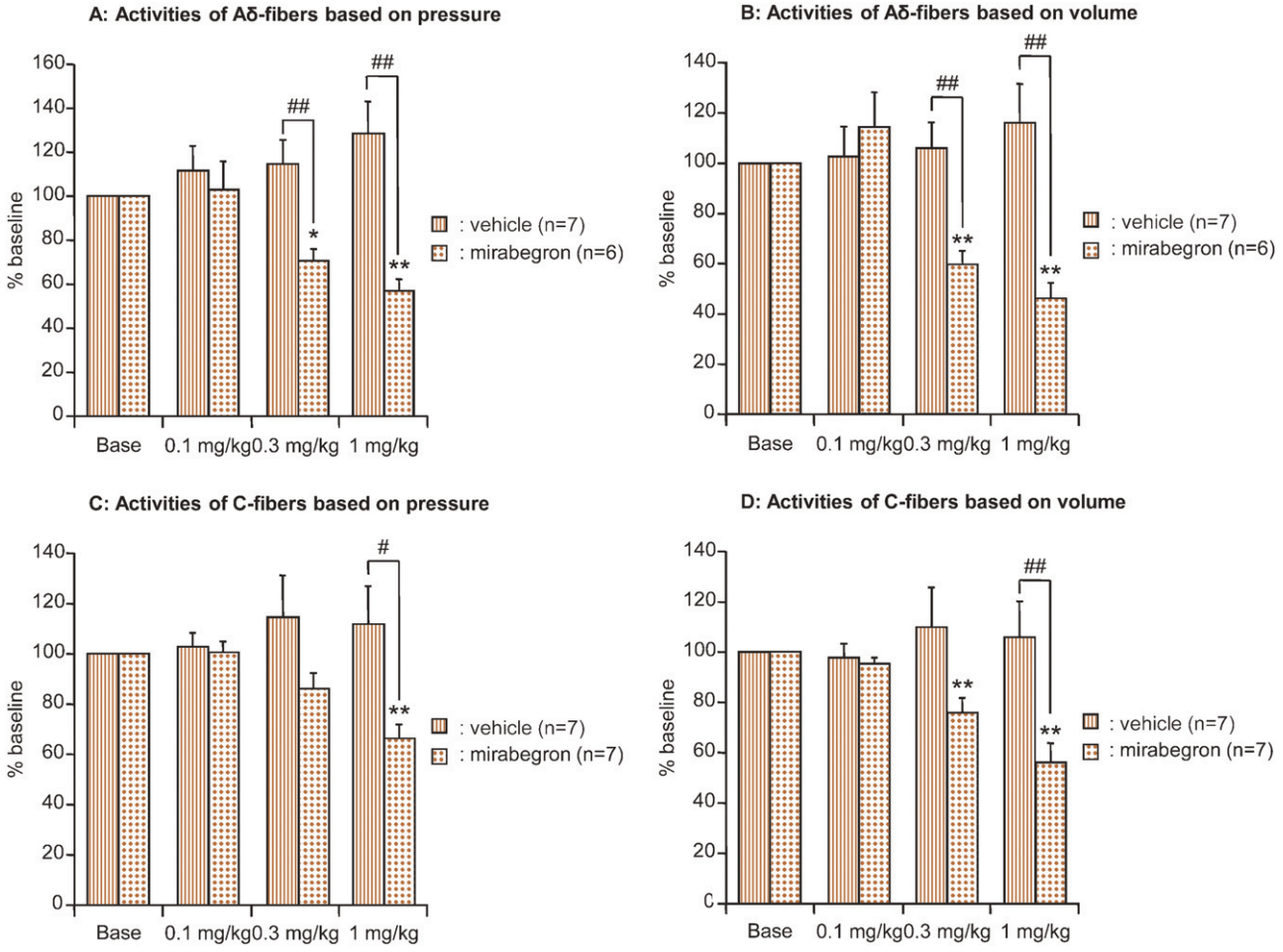

Fig. 3 – Responses to intravenous administration of mirabegron of the (A and B) Aδ-fibers and (C and D) C-fibers integrated during the whole filling phase. The values are expressed as a percentage of baseline activity (mean ± standard error of the mean). The inhibitory effects of mirabegron on both afferent activities were more pronounced when analyzed based on volume changes than when analyzed based on pressure changes. $^{*}p < 0.05$; $^{**}p < 0.01$: significant difference from baseline (two-way analysis of variance followed by the Tukey test); $^{\#}p < 0.05$; $^{\#\#}p < 0.01$: significant difference between vehicle and mirabegron administration (unpaired Student *t* test).

3.4. Effects of mirabegron on Aδ-fiber and C-fiber activities during an isovolumetric condition (n = 40)

There was no significant difference between the baseline of vehicle- and drug-administration regarding the reproducibility/variability of the microcontractions or afferent activities during an isovolumetric condition. In such a condition, the mean bladder pressure and both number and amplitude of microcontractions were significantly decreased after mirabegron administration (except bladder pressure and amplitude of microcontractions at a dose of 0.3 mg/kg, $n = 28$), whereas these parameters did not change with oxybutynin administration (Fig. 4, 5, 6A–6C; $n = 12$). Mechanosensitive single afferent activities of Aδ-fibers were significantly decreased by mirabegron administration at both the 0.3- and 1-mg/kg dose levels, whereas afferent activities of C-fibers were significantly decreased only at 1 mg/kg (Fig. 4, 6D, 6E). In contrast, oxybutynin did not significantly alter either Aδ-fiber or C-fiber afferent activities (Fig. 5, 6D, 6E).

4. Discussion

The results of the present first experiment in response to bladder filling demonstrated that mirabegron, a novel β3-AR agonist, inhibited the afferent activities of both Aδ-fibers and C-fibers in a dose-dependent manner that was more remarkable for Aδ-fibers than C-fibers. These findings are partly consistent with our previous study, which demonstrated that the β3-AR agonist CL316,243 inhibits Aδ-fiber activity elicited by stimulation of bladder distention in similar experimental conditions [18]. In that previous study, however, we examined only one dose of CL316,243, and no such effect was seen on C-fibers with that dose. Nevertheless, the present study suggests that β3-AR agonists can inhibit mechanosensitive C-fiber if its dose is increased. We have found a transient mild decrease in blood pressure (approximately −7 to −8 mm Hg from the baseline) and an increase in heart rate (approximately +10 to +12% from the baseline) after mirabegron administration (1 mg/kg) in urethane-anesthetized rats (unpublished data,

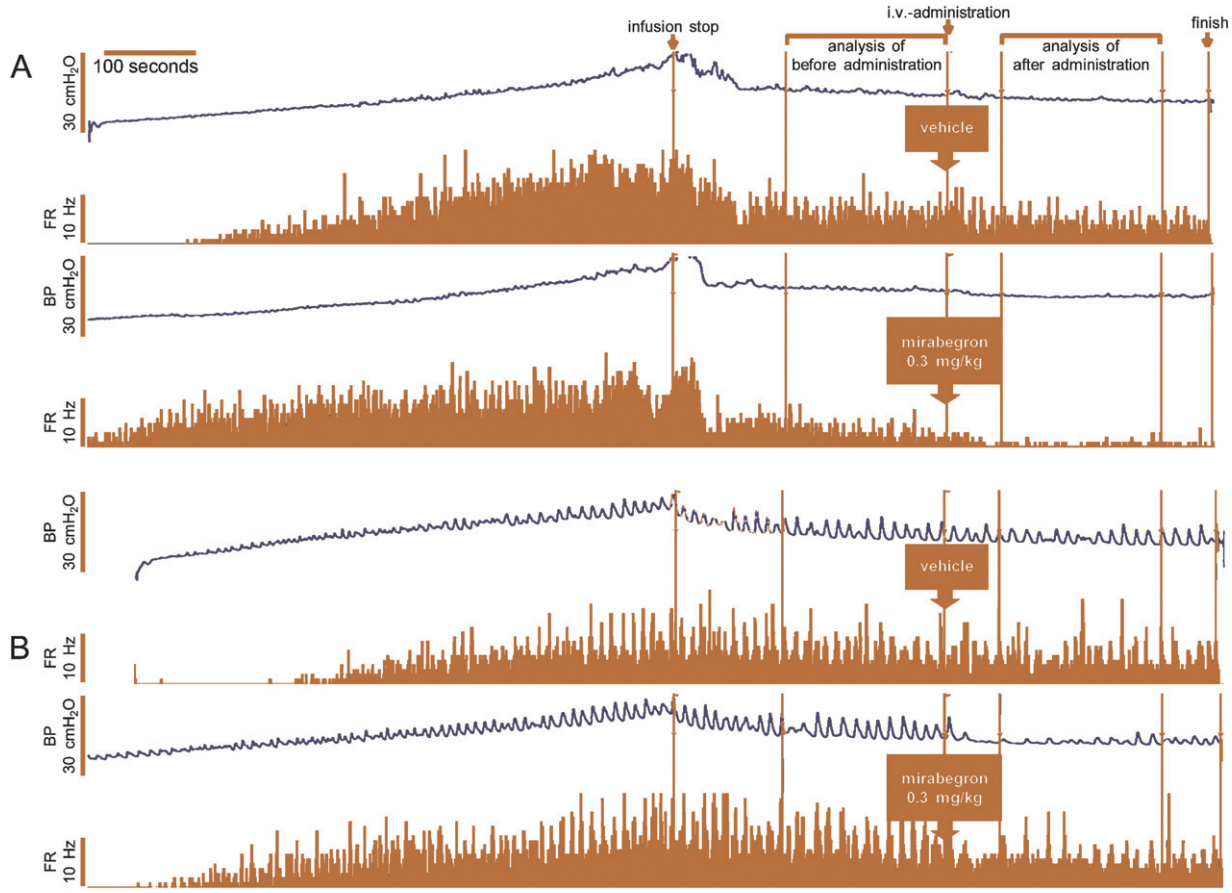

Fig. 4 – Representative recordings of bladder pressure (BP) and firing rate (FR) of (A) Aδ-fiber and (B) C-fiber afferent activity during an isovolumetric condition before and after vehicle or mirabegron (0.3 mg/kg) administration. Mechanosensitive single afferent activities of Aδ-fibers, but not C-fibers, were significantly decreased by mirabegron administration, and such effects appeared to synchronize with the decrease in fluctuation in bladder pressure. IV = intravenous.

see Appendix A). Although we cannot deny an indirect influence on bladder function of these cardiovascular changes with a high-dose administration of mirabegron, mirabegron's action may be due to an action on a part of the urothelial afferent transduction system or a direct action on afferent nerves themselves, as the bladder compliance was not significantly increased. In this first experiment, the inhibition of afferent activities appeared to synchronize with a decrease in bladder microcontractions during filling. To further evaluate the possible relationship between microcontractions and afferent activities in response to mirabegron administration, we made the second experiments under an isovolumetric condition, investigating direct effects of mirabegron on bladder microcontractions and mechanosensitive afferent activities, and compared the results with those of the anticholinergic agent oxybutynin.

The results of this second experiment showed that mirabegron inhibited both bladder microcontractions and Aδ-fiber activity at doses that did not decrease bladder pressure. This finding suggests that the microcontractions may be linked to the mechanosensitive afferent activities of Aδ-fiber and that mirabegron inhibits the afferent activities through suppression of the microcontractions. The microcontractions observed in the present study are of myogenic origin, as no reflex arc through the L6 dorsal roots was preserved in the experimental setup. At higher doses, which also decreased bladder pressure, mirabegron inhibited C-fiber activity, suggesting their linkage. These dose-dependent effects of mirabegron on mechanosensitive afferent activities were consistent with the results of afferent activities in response to bladder filling (the first experiment). In contrast, oxybutynin did not alter either bladder microcontractions or mechanosensitive afferent activities. This dose of oxybutynin (1 mg/kg) is known to significantly decrease the amplitude of rhythmic bladder contractions in urethane-anesthetized rats, presumably by blockade of muscarinic M3 receptors in bladder smooth muscle [9]. Although we have not investigated the effect of oxybutynin on the mechanosensitive afferent activities in response to bladder filling, previous studies showed that oxybutynin (a non–subtype-selective anticholinergic agent) and darifenacin (an M3-selective anticholinergic agent) can inhibit the mechanosensitive afferent activities of both Aδ-fibers and C-fibers ≥30 min after drug administration [1–3]. These different findings on oxybutynin's effect on afferent activities between previous

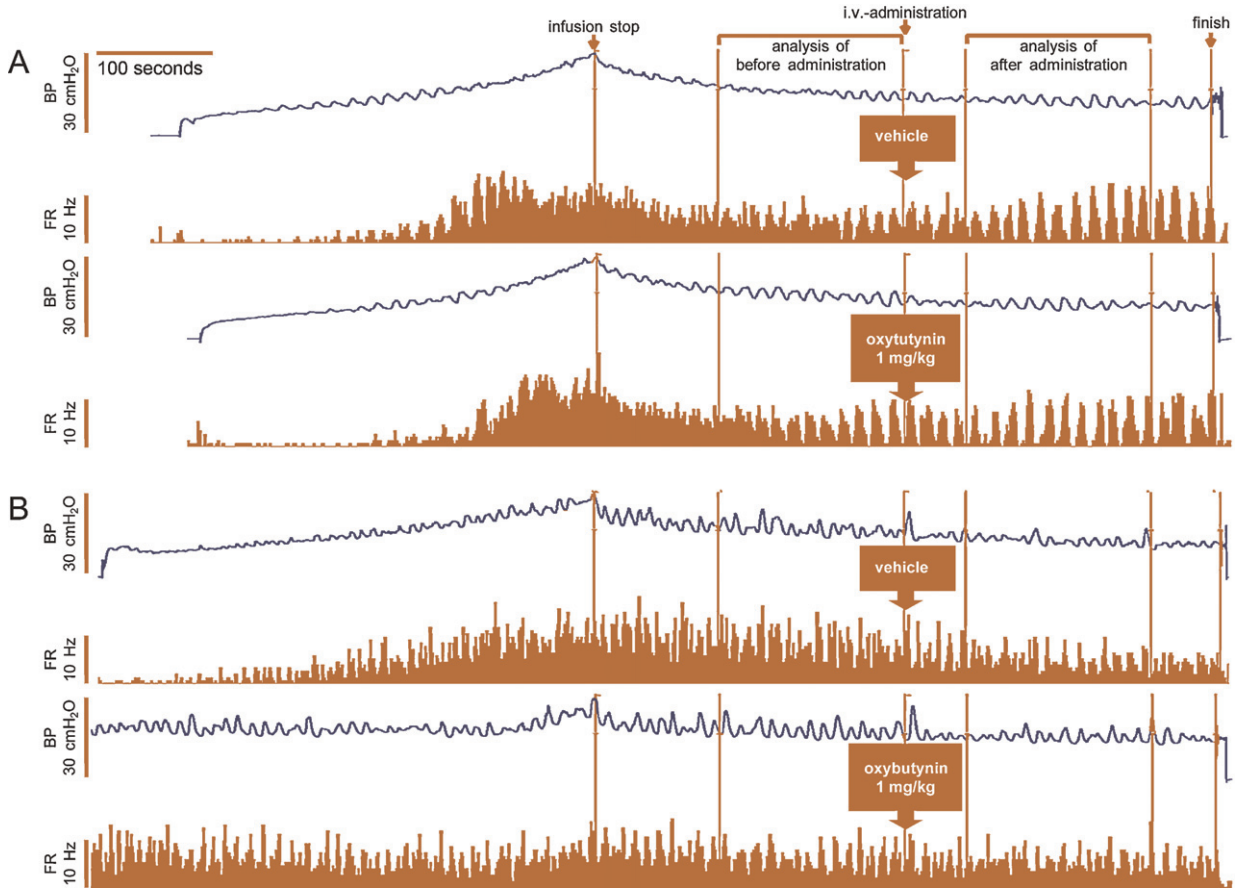

Fig. 5 – Representative recordings of bladder pressure (BP) and firing rate (FR) of (A) Aδ-fiber and (B) C-fiber afferent activity during an isovolumetric condition before and after vehicle or oxybutynin (1 mg/kg) administration. Oxybutynin did not significantly alter either cystometric parameters (bladder pressure and microcontractions) or both afferent activities. IV = intravenous.

studies (during constant filling) and the second experiment of the present study (during an isovolumetric condition) may have occurred because of the difference in evaluation timing (for a 3-min period immediately after intravenous administration compared with 30, 60, 90, 120, and 150 min after subcutaneous or intravenous administration) or the difference in the experimental condition (isovolumetric condition compared with constant bladder filling), which also may contribute to the bladder microcontractions.

In the bladder, Aδ-fibers are located primarily within the detrusor smooth muscle layer, whereas C-fibers are more widespread can be found not only in the detrusor but also in the lamina propria, and often directly adjacent to the urothelial cells themselves [17,27–29]. These morphologic findings regarding the distribution of afferent fibers may support the present functional finding that bladder microcontractions are related to mechanosensitive afferent activity of Aδ-fibers rather than C-fibers. Drake et al. (2003) proposed that localized contractions may stimulate afferent activity, serving to inform the central nervous system about intravesical volume, particularly since neighboring modules are seen to stretch in the guinea pig bladder [21]. In addition, another study by Drake et al. showed that the increased coordination of microcontractions with stretch and the consequent enhancement of intravesical pressure fluctuations are seen in rats following BOO [23]. These intravesical pressure fluctuations observed in BOO rats are known to be suppressed by the β3-AR agonist CL316,243 [19]. Thus, it is likely that β3-AR agonists suppress the afferent activities of Aδ-fibers originating from the bladder wall through the inhibition of bladder microcontractions. It has been reported that β-AR activates the adenylate cyclase pathway, leading to an increase in intracellular Ca^{2+} concentration that, in turn, triggers nitric oxide production and its release in the urothelial cells of the rat bladder [13,14]. In addition, the other study demonstrated that contractile responses to carbachol were inhibited by stimulating urothelial β-ARs, leading to the conclusion that β-AR agonists might stimulate the urothelial release of an unidentified factor that inhibits contractions of the detrusor smooth muscle [15]. More recently, Kurizaki et al. showed decreased expression of bladder mucosal β3-AR mRNA in patients with severe BOO [30]. Taken together, these factors and mechanisms may contribute to the bladder microcontractions and afferent activities, although we need further investigation.

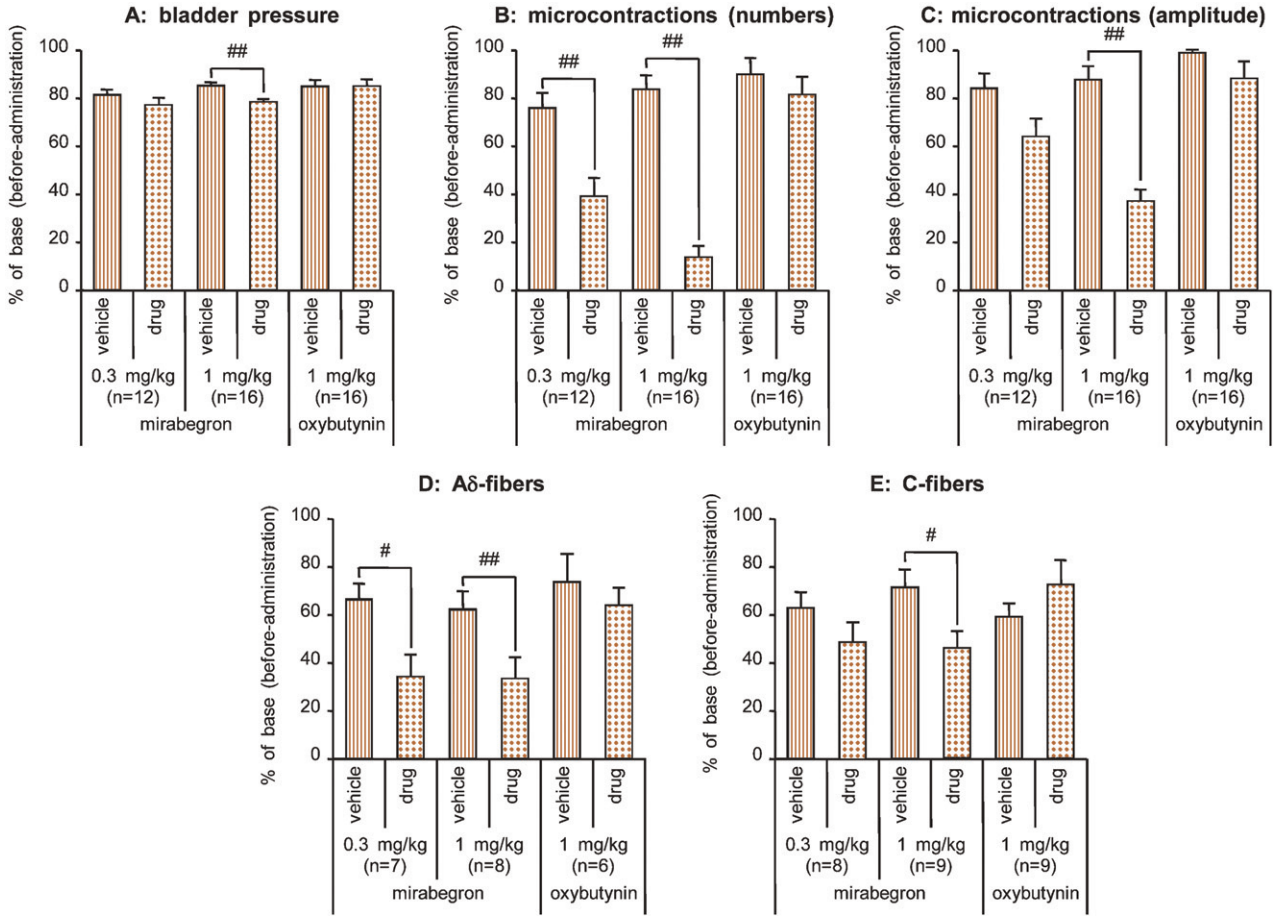

Fig. 6 – Comparative results of (A) mean bladder pressure, (B) number of microcontractions, (C) amplitude of microcontractions, (D) afferent activities of Aδ-fibers, and (E) afferent activities of C-fibers between vehicle and mirabegron or oxybutynin administration. The values were represented as percentages of baseline values and compared between vehicle and drug administration. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; significant differences after vehicle compared with drug administration (unpaired student *t* test).

5. Conclusions

A novel β3-AR agonist, mirabegron, decreased the mechanosensitive afferent activity of both Aδ-fiber and C-fiber in a dose-dependent manner that was more remarkable for Aδ-fiber. In addition, mirabegron can inhibit both bladder microcontractions and Aδ-fiber activity at doses that do not decrease bladder pressure. At higher doses, which also decreased bladder pressure, mirabegron inhibited C-fiber activity. These effects were not observed with oxybutynin. These findings suggest a possible additional action of β3-AR agonists as therapeutic agents for OAB or other bladder sensory disorders.

Author contributions: Yasuhiko Igawa had 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.

Study concept and design: Aizawa, Igawa.
Acquisition of data: Aizawa.
Analysis and interpretation of data: Aizawa, Igawa.
Drafting of the manuscript: Aizawa.
Critical revision of the manuscript for important intellectual content: Homma, Igawa.
Statistical analysis: Aizawa.
Obtaining funding: Igawa, Aizawa.
Administrative, technical, or material support: Igawa.
Supervision: Homma, Igawa.
Other (specify): None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2012.08.056.

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