Naunyn-Schmiedeberg's Arch Pharmacol (2013) 386:71–78
DOI 10.1007/s00210-012-0814-3

ORIGINAL ARTICLE

Effect of mirabegron, a novel β_3-adrenoceptor agonist, on bladder function during storage phase in rats

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Received: 10 August 2012 / Accepted: 12 November 2012 / Published online: 9 December 2012

Abstract Mirabegron, a selective β_3-adrenoceptor agonist, facilitates urine storage function by exerting a relaxing effect on bladder smooth muscle. Here, we investigated the effect of mirabegron on bladder function during the storage phase. We assessed the effect of mirabegron on the resting intravesical pressure in anesthetized rats and also tested antimuscarinics (oxybutynin and tolterodine) under the same experimental conditions. Mirabegron dose-dependently decreased the resting intravesical pressure, while oxybutynin and tolterodine showed no statistically significant effects on resting intravesical pressure. We also investigated the effect of mirabegron on bladder function using cystometry technique in conscious rats with bladder outlet obstruction. While mirabegron dose-dependently decreased the frequency of nonvoiding contractions, considered an index of abnormal response in bladder storage, no significant effects were noted on the amplitude of nonvoiding contractions, micturition pressure, threshold pressure, voided volume, residual volume, or bladder capacity. Neither oxybutynin nor tolterodine affected the frequency of nonvoiding contractions; however, oxybutynin increased residual volume and tended to decrease voided volume in a dose-dependent manner, and tolterodine dose-dependently decreased voided volume. Taken together, these results shed light on the suggestion of mirabegron as a therapeutic agent, compared with antimuscarinics, with its most prominent effect being the facilitation of bladder storage.

Keywords Mirabegron · β_3-adrenoceptor · Overactive bladder · Storage phase · Bladder outlet obstruction

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Introduction

During storage and voiding phases, the bladder is regulated by a complex neural control system, involving alternate sympathetic and parasympathetic input. During bladder filling, activation of sympathetic nerve projected to bladder smooth muscle results in β-adrenoceptor-mediated relaxation (Andersson and Wein 2004). β-Adrenoceptors are subclassified into three β-adrenoceptor subtypes, β_1-, β_2-, and β_3-adrenoceptors, and relaxant effects induced by activation of each subtype have been reported to vary among species in mammalians (Yamazaki et al. 1998). While the main subtype of mRNA expression among β-adrenoceptor subtypes in human bladder smooth muscle is β_3-adrenoceptor, both β_3- and β_2-adrenoceptors contribute to bladder relaxation in rats (Michel and Vrydag 2006). Given the important roles that these receptors play in relaxation and improvement of compliance of the mammalian bladder, previous studies have suggested that activation of bladder β_3-adrenoceptors may be of therapeutic relevance to the treatment of overactive bladder conditions (Michel and Vrydag 2006). In pharmacological experiments, it has been reported that agonists for rat β-adrenoceptors, such as CL-316243 and BRL37344A, increased bladder capacity in rats (Takeda et al. 2000; Woods et al. 2001).

Overactive bladder is characterized by symptoms of frequency and urgency with or without urge incontinence (Abrams et al. 2002) and has a profound negative impact on the quality of life of those affected. With regard to the pharmacotherapy, muscarinic receptor antagonists are currently used for the treatment of this disorder. However, although the effectiveness of available drugs such as oxybutynin and later-introduced muscarinic receptor antagonists has been demonstrated in several clinical studies, the relatively high incidence of adverse events such as dry mouth, constipation, and blurred vision often leads to

treatment discontinuation (Moore et al. 1990; Thuroff et al. 1991; Athanasopoulos and Giannitsas 2011), highlighting the medical need for the development of new drugs with a better balance between efficacy and tolerability. Mirabegron is a selective β_3-adrenoceptor agonist developed for the treatment of overactive bladder. Mirabegron increased cyclic AMP accumulation with full activity at human β_3-adrenoceptor, whereas it showed little effect at human β_1- or β_2-adrenoceptors (Takasu et al. 2007). Mirabegron is confirmed to relax bladder muscle isolated from human and rats (Takasu et al. 2007).

Michel and Sand (2009) showed that isoproterenol, a nonselective β-adrenoceptor agonist, exerted a relaxant effect with a pEC_{50} value of 8.76 under a condition of passive tension (10 mN) versus 7.27 with precontraction induced by a submaximal concentration of carbachol (1 μmol/L), indicating that isoproterenol functioned more effectively under conditions closely similar to the natural storage phase than under conditions of substantial voiding stimulation. Taken together, these data suggest that β-adrenoceptor agonists would be specifically active during the bladder storage phase rather than the voiding phase.

In the present study, we investigated the effect of mirabegron on resting intravesical pressure in anesthetized rats and compared it with the effects of antimuscarinics (oxybutynin and tolterodine). We also examined the effect of mirabegron on bladder function, particularly focusing on nonvoiding contraction, considered an index of abnormal response in bladder storage, in rats with bladder outlet obstruction and compared the findings with those for antimuscarinics. Especially, bladder outlet obstructed rats were used as a detrusor overactivity model in the present study.

Materials and methods

Drugs

Mirabegron and tolterodine tartrate were synthesized by Astellas Pharma Inc. (Tokyo, Japan). Oxybutynin chloride was obtained from Sigma-Aldrich (St. Louis, MO, USA). The maximum doses of drugs used in the present study were dissolved with physiological saline containing 5 % Cremophor® EL (Nacalai Tesque, Kyoto, Japan) and 10 % *N*,*N*-dimethylacetamide (Junsei Chemical Co., Ltd., Tokyo, Japan) and diluted with physiological saline to make lower doses of the agents.

Animals

Female Wistar rats were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). All animal experiments were performed in compliance with the International Guiding Principles for Biomedical Research Involving Animals. The protocol for this study was approved by the Animal Ethics Committee of Astellas Pharma Inc.

Resting intravesical pressure in rats

Thirty rats weighing 245–310 g were divided into five groups and anesthetized with pentobarbital (50 mg/kg) administered intraperitoneally (i.p.). Pentobarbital was used in order to suppress the micturition reflex during drug evaluation. Through the incisions at dorsal–lateral parts of the rats, the ureters on both sides were tied with ligatures and cut at the proximal end, after which the incisions were sutured with wound clips. A polyethylene catheter (PE-50; Becton, Dickinson & Co., Franklin Lakes, NJ, USA) was then inserted into the bladder via the external urethral orifice and bound around the urethra, and any urine in the bladder was removed through the catheter by gently pressing on the abdomen. The bladder catheter was then connected to a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan) and a syringe filled with physiological saline through a three-way connector. Intravesical pressure was measured using a pressure amplifier (AP-601G; Nihon Kohden) connected to a pressure transducer. Another catheter (PE-50) was inserted into the left carotid artery and connected to a pressure transducer (TP-400T, Nihon Kohden) to measure blood pressure. Animal condition was monitored via blood pressure during the experiment. For intravenous (i.v.) administration of drugs or the vehicle, a polyethylene catheter (PE-50) with a needle tip was inserted into the femoral vein. The mean threshold bladder pressure which evokes the distention-induced micturition reflex has been reported to be 8.2–13.6 cmH_2O in anesthetized rats (Conley et al. 2001; Lecci et al. 2000; Read et al. 2003). In this study, initial intravesical pressure was set to 6 cmH_2O, a value below the threshold of micturition, to represent the storage phase by gradual instillation of physiological saline into the bladder. After a stabilization period of 5 min, rats in each of the five treatment groups received physiological saline, mirabegron (0.003, 0.03, 0.3, and 3 mg/kg), oxybutynin (0.001, 0.01, 0.1, and 1 mg/kg), or tolterodine (0.0003, 0.003, 0.03, and 0.3 mg/kg) at a volume of 1 mL/kg body weight intravenously at increasing doses at 5-min intervals.

Bladder function in rats with bladder outlet obstruction

Preparation of partial urethral obstruction

Female rats were used for pragmatic (less complicated surgery) rather than scientific reasons. Rats weighing 160–250 g were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), in order to suppress the micturition reflex during drug evaluation. A polyethylene catheter (PE-20;

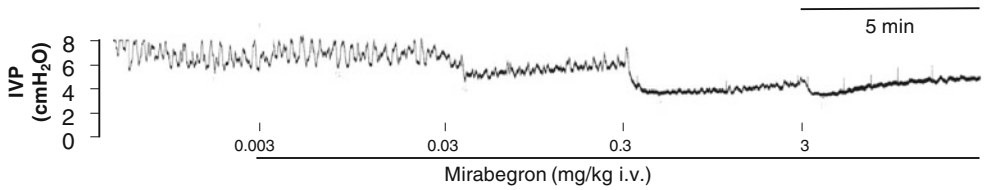

Fig. 1 A typical trace of intravesical pressure (IVP) during the storage phase in anesthetized rats. The baseline intravesical pressure was adjusted to about 6 cmH_2O, as evaluation of the effect of drugs on intravesical pressure at state corresponding to the storage phase

Becton, Dickinson & Co.) with an outside diameter of 1.09 mm was inserted into the bladder via the external urethral orifice. The abdomen was opened through a midline incision, and the urinary bladder and urethra were exposed. Two silk ligatures (5–0) were placed around the proximal urethra and tied in the presence of an intraluminal indwelling catheter. Sham-operated rats underwent similar procedures without partial urethral obstruction. After removing the catheter from the urethra, the abdominal wall was sutured, and ampicillin sodium (150 mg/kg) was administered subcutaneously (s.c.). These animals were all housed with free access to food and water and allowed to recover.

Catheterization for a jugular vein and urinary bladder

Thirty rats with bladder outlet obstruction were divided into five groups and were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) 11–12 days after operation of partial urethral obstruction. Given that urinary bladder with 6-week obstruction exhibits markedly more severe hypertrophy than bladder after 11–12-day obstruction, we choose the 11–12-day obstruction model for evaluation of drug activity. The urinary bladder and urethra were exposed via a middle incision made in the abdominal wall and gently freed from adhering tissues. After removing the silk ligatures tying the urethra, a polyethylene catheter (PE-50) was implanted in the bladder through its apex. For intravenous bolus injection, another polyethylene catheter (PE-50) filled with physiological saline containing sodium heparin was inserted into a jugular vein. These catheters were tunneled subcutaneously and anchored to the skin of the back of the neck with silk ligatures, after which ampicillin sodium (150 mg/kg, s.c.) was administered. The free ends of the catheters were then sealed, and each rat was housed in an individual cage with free access to food and water and allowed to recover.

Cystometric investigation

Cystometric investigation was performed in conscious animals 3 days after bladder catheterization. Conscious rats deprived of water overnight were placed in Bollman's cages. The bladder catheter was connected to a pressure transducer (TP-400 T; Nihon Kohden) and a syringe pump (STC-525; Terumo, Tokyo, Japan) via a three-way connecter. Physiological saline at room temperature was continuously infused into the bladder through the bladder catheter at rates of 5–15 mL/h in the bladder outlet obstructed rats and 3.6 mL/h in sham-operated rats to maintain micturition intervals of 20–40 min, in an effort to keep drug evaluation times fairly constant. Intravesical pressure was measured using a pressure amplifier (AP-601G; Nihon Kohden) connected to a pressure transducer. Following a stabilization period at least 3 h, the bladder was emptied by draining urine through the bladder catheter, and then physiological saline was continuously infused again into the bladder. The average values of the cystometric parameters (voided volume [volume of expelled urine estimated from the urinary weight, assuming the relative density of urine to be 1], residual volume [bladder capacity minus voided volume], bladder capacity [residual volume at the latest previous micturition plus volume of infused physiological saline], micturition pressure [maximum intravesical pressure during micturition], threshold pressure [intravesical pressure immediately prior to micturition], and nonvoiding contractile activity [mean amplitude and frequency of intravesical pressure

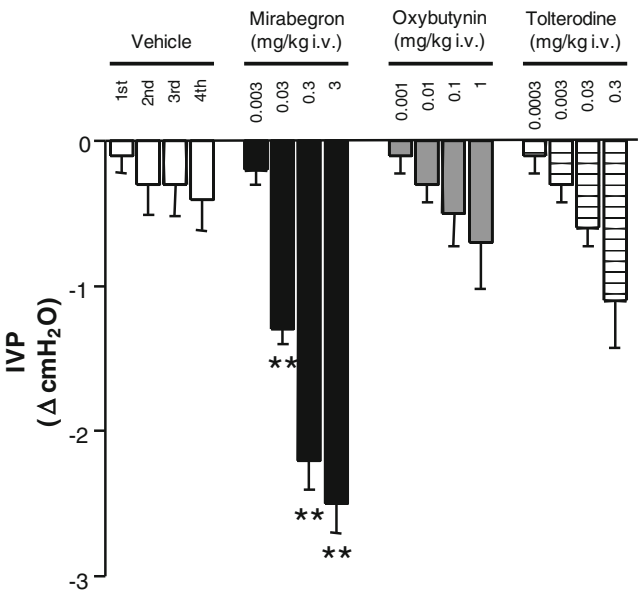

Fig. 2 Effects of mirabegron, oxybutynin, and tolterodine on intravesical pressure (IVP) in anesthetized rats. Drugs are administered intravenously at increasing doses. Each column represents the mean± S.E.M. of change from pre-value of six rats. **denote $P<0.01$; significant difference from the corresponding value in the vehicle-treated group (Student's t test)

fluctuations of 2 cmH_2O or more during 10 min prior to micturition]) of two micturition cycles just before the first injection of test substances were defined as the baseline. Afterward, rats in each of the five treatment groups received physiological saline, mirabegron (0.1, 0.3, 1, and 3 mg/kg), oxybutynin (0.03, 0.1, 0.3, and 1 mg/kg), or tolterodine (0.01, 0.03, 0.1, and 0.3 mg/kg) at a volume of 1 mL/kg body weight were intravenously administered at increasing doses at intervals of approximately 20–40 min. The evaluation was performed for each cystometric parameter of one reproducible micturition cycle after injection of drug. After investigation completion, urinary bladders were isolated from both sham-operated and bladder outlet obstructed rats and weighed.

Statistical Analysis

Data are expressed as the mean±S.E.M. of five to six animals. Statistical significance in baseline values of cystometric parameters between sham-operated and bladder outlet obstructed rats were analyzed using Student's *t* test. Statistical significance in baseline values of cystometric parameters among vehicle-treated and drug-treated rats with bladder outlet obstruction was analyzed using one-way analysis of variance. Statistical significance between the corresponding values in the time-matched vehicle-treated group and drug-treated group were analyzed using Student's *t* test. $P<0.05$ was considered statistically significant. All data analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

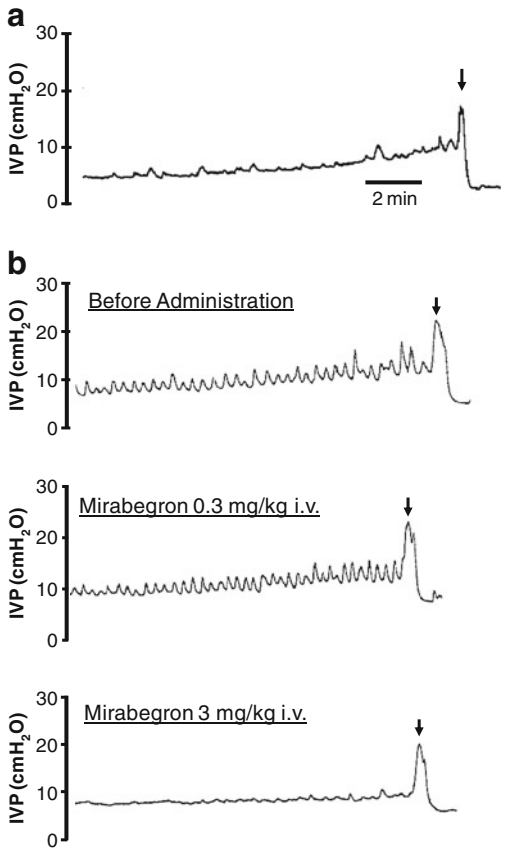

Fig. 3 Typical traces of cystometrogram in conscious sham-operated (**a**) and mirabegron-treated bladder outlet obstructed rats (**b**). Saline was continuously infused into the bladder at rates of 3.6 and 5 mL/h in sham-operated and bladder outlet obstructed rats, respectively. *Arrows* indicate micturition

Results

Intravesical pressure during urine storage phase in rats

A typical trace of intravesical pressure during storage phase is shown in Fig. 1. No significant differences were noted in baseline values of intravesical pressure between the vehicle- and drug-treated groups using one-way analysis of variance. Mirabegron (0.003–3 mg/kg i.v.) dose-dependently decreased the intravesical pressure, an effect which was statistically significant at doses of 0.03 mg/kg i.v. or more. In contrast, neither oxybutynin (0.001–1 mg/kg i.v.) nor tolterodine (0.0003–0.3 mg/kg i.v.) exerted any significant effects on the intravesical pressure (Fig. 2). In addition, mirabegron dose-dependently diminished spontaneous bladder contractions (Fig. 1), whereas oxybutynin and tolterodine showed little effects.

An 11 % increase in heart rate and a 29 % decrease in mean blood pressure from pre-values were observed at 3 mg/kg i.v.; the dose was highest dose we tested, in the mirabegron-treated group. At doses of 0.03 and 0.3 mg/kg i.v., started to find a statistical significance in efficacy,

Table 1 Comparison of cystometric parameters in conscious sham-operated and bladder outlet obstructed rats

	Sham	BOO
Frequency of nonvoiding contraction (times/min)	0.33±0.09	2.4±0.3**
Amplitude of nonvoiding contraction (cmH_2O)	2.6±0.3	3.6±0.3*
Micturition pressure (cmH_2O)	28±3	25±3
Threshold pressure (cmH_2O)	15±5	8.2±1
Voiding volume (mL)	1.7±0.08	3.5±0.7*
Residual volume (mL)	0.04±0.01	1.3±0.7
Bladder capacity (mL)	1.8±0.09	4.8±1*
Bladder weight (mg/100 g body weight)	53±4	190±20**

The comparison of baseline values was analyzed between sham-operated rats and those of bladder outlet obstructed rats. Each value represents the mean±S.E.M. of six rats

*$P<0.05$; **$P<0.01$; significantly different from the value of sham-operated group (Student's *t* test)

little effect on heart rate (2–7 % increase) or blood pressure (4–6 % decrease) was observed; other drugs showed little effects on both parameters, and also little effects on heart rate (2 % increase) and blood pressure (2–5 % decrease) from pre-values were observed in the vehicle-treated group.

Bladder functions in rats with bladder outlet obstruction

Typical traces of sham-operated and bladder outlet obstructed rats are shown in Fig. 3a, b. Cystometoric parameters of sham-operated and bladder outlet obstructed rats are shown in Table 1. In the obstructed rats, frequency and amplitude of nonvoiding contractions, voided volume, bladder capacity, and bladder weight were significantly increased compared with values in sham-operated rats (Student's *t* test). There were no significant differences in baseline values of cystometric parameters between the vehicle- and drug-treated groups using one-way analysis of variance (data not shown). Mirabegron (0.1–3 mg/kg i.v.) dose-dependently reduced the frequency of nonvoiding contractions (Fig. 3b), with significant effects noted at doses of 1 mg/kg i.v. or more (Fig. 4) and a dose of 3 mg/kg i.v. almost completely diminished them. In contrast,

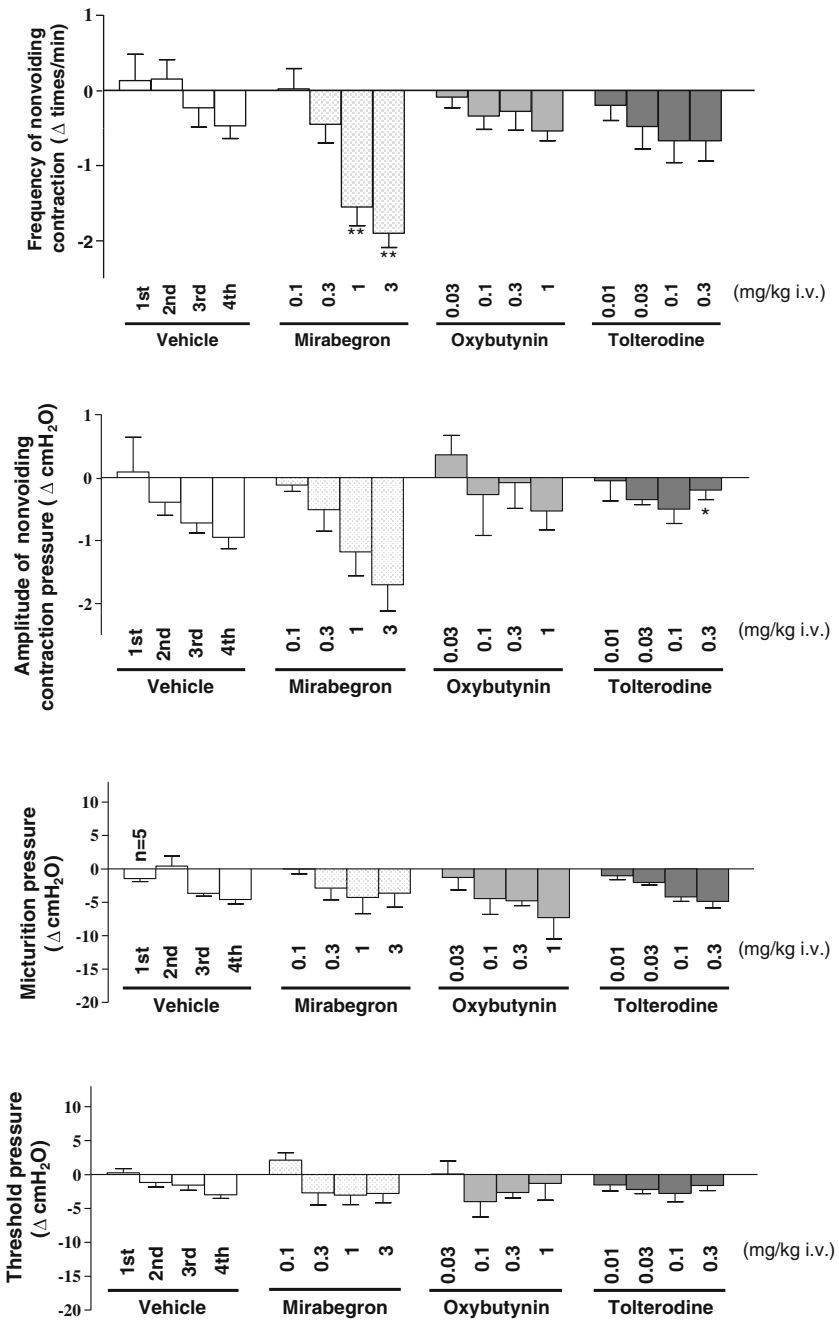

Fig. 4 Effects of mirabegron, oxybutynin, and tolterodine on frequency and amplitude of nonvoiding contractions, micturition pressure, and threshold pressure in bladder outlet obstructed rats. Each column represents the mean± S.E.M. of change from pre-value of five to six rats. * $P<0.05$, ** $P<0.01$; significant difference from the value of corresponding vehicle-treated group (Student's *t* test)

oxybutynin (0.03–1 mg/kg i.v.) and tolterodine (0.01–0.3 mg/kg i.v.) did not affect frequency of nonvoiding contractions (Fig. 4). Mirabegron exerted no remarkable effects on micturition pressure, threshold pressure, residual volume, voided volume, or bladder capacity up to 3 mg/kg i.v., whereas amplitude of nonvoiding contractions were dose-dependently decreased with no statistical difference (Fig. 4). Oxybutynin increased residual volume and tended to decrease voided volume in a dose-dependent manner (Fig. 5). Tolterodine decreased voided volume in a dose-dependent manner (Fig. 5).

Discussion

In the present study, mirabegron significantly decreased the resting intravesical pressure in anesthetized rats, whereas oxybutynin and tolterodine did not decrease it. Isoproterenol and a β_3-adrenoceptor agonist CL316,243 have been reported to exert effects similar to those achieved in our study here, where fluctuations in intravesical pressure were gradually diminished, thereby reducing intravesical pressure, whereas atropine showed little effects in rats (Takeda et al. 2000). Although fluctuations in intravesical pressure noted in the present experimental setup may not always indicate detrusor overactivity, diminution or decrease in fluctuations of intravesical pressure is a demonstrated effect of β_3-adrenoceptor agonists and suggests amelioration of detrusor overactivity. Given that antimuscarinics have been shown to exert less obvious effects on the intravesical pressure during the storage phase, β_3-adrenoceptor plays an important role in bladder relaxation and that β_3-adrenoceptor agonists can induce relaxation during storage phase. Taken together, these present and previous findings indicate that β_3-adrenoceptor agonists have an advantage over antimuscarinics in improving storage function.

An 11 % increase in heart rate and a 29 % decrease in mean blood pressure from pre-values were observed at 3 mg/kg i.v.; the dose was highest dose we tested, in the mirabegron-treated group. At doses of 0.03 and 0.3 mg/kg i.v., started to find a statistically significance in efficacy, little effect on heart rate (2–7 % increase) or blood pressure (4–6 % decrease) was observed; other drugs showed little effects on both parameters and also little effects on heart rate (2 % increase) and blood pressure (2–5 % decrease) from pre-values were observed in the vehicle-treated group. Moreover, a possibility that the effects of mirabegron on cardiovascular systems affects the efficacy in bladder may be denied because the effects of mirabegron on cardiovascular systems at doses which the

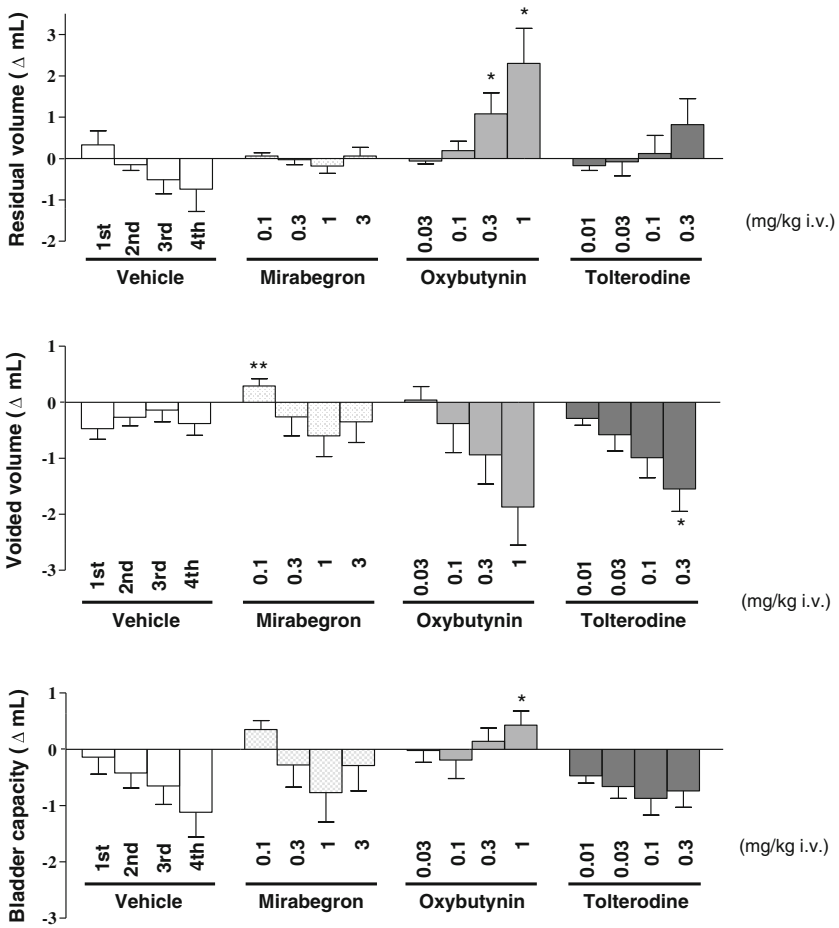

Fig. 5 Effects of mirabegron, oxybutynin, and tolterodine on residual volume, voided volume, and bladder capacity in bladder outlet obstructed rats. Each column represents the mean±S.E.M. of change from pre-value of six rats. * $P<0.05$, ** $P<0.01$; significant difference from the value of corresponding vehicle-treated group (Student's t test)

efficacy of mirabegron starts to find a statistically significance in bladder are similar to those of vehicle.

Bladder outlet obstruction associated with benign prostatic hyperplasia is known to induce detrusor overactivity and voiding dysfunction. Lower urinary tract symptoms associated with bladder outlet obstruction therefore comprise a combination of storage symptoms, including urinary frequency and urgency, as well as voiding symptoms such as hesitancy and slow stream. These storage symptoms may be associated with increased contractile activity in the detrusor muscle during the storage phase. Previous investigations of bladder function in bladder outlet obstructed rats have shown a condition which shares similarities to that seen in patients with detrusor overactivity (Guarneri et al. 1991; Igawa et al. 1994).

In this study, mirabegron decreased the number of nonvoiding contractions but not their amplitude so much induced by bladder outlet obstruction, suggesting that mirabegron may involve on/off switch of nonvoiding contractions. The origin of nonvoiding contractions is believed to involve myogenic and afferent activity components (Gillespie et al. 2012), both of which would be affected by β_3-adrenoceptor agonists (Gillespie et al. 2012), thereby facilitating bladder extension (decrease of urinary frequency) and suppressing overstimulation of the micturition reflex. Recently, it has been reported that β_3-adrenoceptor agonists affect the afferent pathways innervating the bladder (Aizawa et al. 2010; Kanai et al. 2011), and Aizawa et al. (2010) showed that a β_3-adrenoceptor agonist (CL316,243) can inhibit prostaglandin E_2-induced C-fiber hyperactivity in anesthetized rats. Further, Aizawa et al. (2012) also demonstrated that mirabegron predominantly inhibits Aδ-fiber activity, but also inhibits C-fiber activity at a high dose. However, such interaction of β-adrenoceptor agonists with afferent pathways may not be appropriately evaluated in the bladder outlet obstructed model, whereas the amelioratory effects of β-adrenoceptor agonists on urgency have already been demonstrated in a clinical setting. In fact, mirabegron has been proven to be clinically effective on urgency in randomized placebo-controlled studies in patients with overactive bladder (Chapple et al. 2010; Khullar et al. 2011; Nitti et al. 2011).

While neither oxybutynin nor tolterodine significantly inhibited nonvoiding contractions in the present study, tolterodine has been previously reported to decrease amplitude and frequency of nonvoiding contractions in bladder outlet obstructed rats (Kaiho et al. 2007; Gillespie et al. 2012). This discrepancy may have been due to differences in evaluation methods or experimental conditions (Gillespie et al. 2012; Kokubun et al. 2010; Moore et al. 2002; Streng et al. 2006).

Although tolterodine showed an inhibitory effect on nonvoiding contractions in 6-week-obstructed rats (Kaiho et al. 2007; Gillespie et al. 2012), voiding efficiency and maximal voiding pressure were also decreased, suggesting potential of tolterodine to induce urinary retention. In contrast, mirabegron exerts an inhibitory effect on nonvoiding contractions independent of the duration of obstruction, without affecting voiding pressure or residual urine volume, and is therefore less likely to induce urinary retention at therapeutic doses, a theoretical advantage over antimuscarinics. In fact, total plasma concentration (AUC_{inf}) by single intravenous administration of 3 mg/kg in rats is higher than the AUC_{inf} by single oral administration of 50 mg mirabegron (the therapeutic dose) in humans (unpublished data) by four-fold to five-fold, which is thought to be a marginal difference. Regarding the mechanism behind mirabegron's lack of any effect on voiding function, the following mechanism is considered. In the urinary bladder, acetylcholine released from parasympathetic nerves during the voiding phase activates postjunctional muscarinic M_2 receptors and inhibits adenylate cyclase activity mediated by β-adrenoceptors. At the same time, acetylcholine also stimulates muscarinic M_3 receptors and activates the phosphatidylinositol-Ca^{2+} recruitment system (Igawa 2000). Hegde et al. (1997) demonstrated that activation of muscarinic M_2 receptors opposes β-adrenoceptor-mediated bladder relaxation both in vitro and in vivo. Relaxation of bladder smooth muscle by mirabegron may therefore be canceled out by muscarinic M_2 receptor activation in the voiding phase. These findings in turn suggest that mirabegron may have a low risk of causing urinary retention. In conclusion, our findings here suggest that mirabegron potently facilitates urine storage function compared with antimuscarinics.

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