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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.