495

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IN VITRO AND IN VIVO EFFECTS OF MIRABEGRON (YM178) ON URINARY BLADDER FUNCTION IN RATS

Hypothesis / aims of study

It is well known that β_3-adrenoceptors (ARs) play a predominant role in bladder relaxation in many species. Mirabegron (YM178), a novel selective β_3-AR agonist, relaxed bladder strips isolated from humans [1] and significantly improved the symptoms in patients with overactive bladder syndrome (OAB) [2]. In the present study, the *in vitro* effects of mirabegron were investigated using Chinese hamster ovary (CHO) cells stably expressing rat β_3-ARs and bladder strips isolated from rats. In addition, to confirm the mechanical differences from antimuscarinics, oxybutynin and tolterodine, the influence of these drugs during the urine filling phase was also investigated using *in vivo* experimental methods described previously [3].

Study design, materials and methods

Agonistic activity for rat β_3-ARs: CHO cells expressing rat β_3-ARs were seeded and incubated at 37 °C in Ham's F-12 medium containing 10% fetal bovine serum, 100 units/mL penicillin G, 100 µg/mL streptomycin sulfate and 500 µg/mL geneticin. Approximately 24 hr after seeding, the culture medium was replaced with serum-free Ham's F-12 medium and incubated for an additional 24 hr. On the day of experiment, mirabegron or isoproterenol, a non-selective β-AR agonist, was incubated with the CHO cells in the presence of 3-isobutyl-1-methylxantine, a phosphodiesterase inhibitor, and the amount of cyclic adenosine monophosphate (cAMP) was determined using the homogeneous time-resolved fluorescence assay with a cAMP kit. The 50% effective concentration (EC_{50}) and intrinsic activity were calculated, taking the maximum response to isoproterenol as 100% (n=4).

Relaxant effect in rat bladder strips: Whole bladders were isolated from male Wistar rats (280–350 g) immediately after euthanasia; bladder strips (3x10 mm) were then prepared in Krebs-Henseleit solution oxygenated with a gas mixture of 95% O_2/5% CO_2 at 37 °C. Each preparation was suspended in an organ bath under a loading tension of 0.5 g. After a stabilization period, relaxant effects of mirabegron or isoproterenol were determined by applying each drug to the bath solution cumulatively at approximately 5 min intervals. To confirm which AR-subtype is involved in bladder relaxation, 100 nmol/L CGP-20712, a selective β_1-AR antagonist, or 100 nmol/L ICI-118551, a selective β_2-AR antagonist, was added 10 min prior to addition of the test drugs. At the end of the experiments, 100 µmol/L papaverine was added to obtain maximal relaxant response. EC_{50} and maximal relaxation rates were calculated (n=3).

Effects on intravesical pressure (IVP) during filling phase in anesthetized rats: Female Wistar rats (245–310 g) were anesthetized with pentobarbital (50 mg/kg, intraperitoneal injection). After their ureters were tied on both sides, a polyethylene catheter (PE-50) was inserted into the bladder via the external urethral orifice and was bound at the urethra by suture. To measure the IVP, the catheter was connected to a pressure transducer. The initial IVP was adjusted to approximately 6 cm H_2O by gradual instillation of physiological saline into the bladder through the catheter. After a stabilization period, either mirabegron or an antimuscarinic (oxybutynin or tolterodine) was intravenously (i.v.) administered at four increasing doses at 5 min intervals. Drug effects were calculated as amount of change from pre-values and student's *t*-tests were done with a time-matched vehicle group (n=6).

Results

Agonistic activity for rat β_3-ARs: In CHO cells stably expressing rat β_3-ARs, mirabegron and isoproterenol induced intracellular cAMP accumulation in a concentration-dependent manner with EC_{50} values of 19 and 60 nmol/L, respectively. The intrinsic activity value of mirabegron was 1.0 when the maximum response to isoproterenol was taken as 1.0.

Relaxant effect in rat bladder strips: Mirabegron and isoproterenol relaxed bladder strips isolated from rats in a concentration-dependent manner. The EC_{50} values of mirabegron and isoproterenol were 290 and 54 nmol/L, respectively. The maximal relaxant rates at 10 µmol/L of each drug were 92.4 and 96.6%, respectively. The isoproterenol concentration response curve shifted rightwards in the presence of ICI-118551 (100 nmol/L), whereas CGP-20712 had no effect even at 100 nmol/L. The mirabegron concentration response curve was not affected by either 100 nmol/L CGP-20712 or ICI-118551.

Effects on IVP during the filling phase in anesthetized rats: There was no significant difference among the baseline pressure of each group before drug dosing (one way ANOVA). As shown in Figure 1, mirabegron (0.003–3 mg/kg i.v.) decreased the IVP in a dose-dependent manner, and the effect was statistically significant at doses ≥ 0.03 mg/kg i.v. ($p<0.01$, Student's *t*-test vs time-matched vehicle group). In comparison, oxybutynin (0.001–1 mg/kg i.v.) and tolterodine (0.0003–0.3 mg/kg i.v.) slightly decreased IVP, but the effects were not significant even at the highest dose.

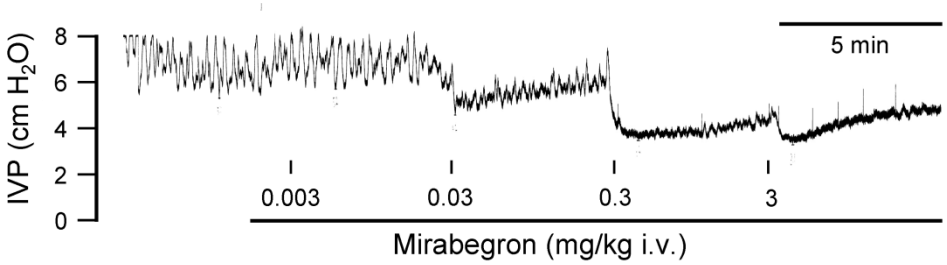

Figure 1. A representative chart of intravesical pressure in anesthetized rats. Mirabegron was intravenously administered at four increasing doses at 5 min intervals. IVP: Intravesical pressure.

Interpretation of results

In the rat urinary filling phase, both β_2- and β_3-ARs appear to be involved in bladder relaxation through adrenergic stimulation. Taken together, our data show that the mirabegron-induced bladder relaxation and decrease in IVP during the filling phase were caused via the stimulation of β_3-ARs. In addition, it has been reported that mirabegron, at the same dose range as that used in the present study, does not affect voiding contractions [1]. In contrast, antimuscarinics, which inhibit voiding contractions, had little effect on the IVP during the filling phase. These data suggest that mirabegron may enhance urine storage function through β_3-AR mediated-bladder relaxation, thus, unlike antimuscarinics, mirabegron has little effect on voiding contractions.

Concluding message

These data suggest that mirabegron may enhance urine storage function at low pressure bladder filling via activation of β_3-AR in the bladder, without affecting voiding contractions

References

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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