European Journal of Pharmacology 699 (2013) 101–105

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

The novel β_3-adrenoceptor agonist mirabegron reduces carbachol-induced contractile activity in detrusor tissue from patients with bladder outflow obstruction with or without detrusor overactivity

Julie Svalø [a], Jørgen Nordling [b], Kirsten Bouchelouche [a], Karl-Erik Andersson [c], Cees Korstanje [d], Pierre Bouchelouche [a,*]

[a] Smooth Muscle Research Center, Department of Clinical Biochemistry, Copenhagen University Hospital at Koege, Lykkebaekvej 1, 4600 Koege, Denmark
[b] Department of Urology, Copenhagen University Hospital at Herlev, Herlev Ringvej 75, 2730 Herlev, Denmark
[c] Wake Forest Institute for Regenerative Medicine, Wake Forest Baptisk Medical Center, N C, USA
[d] Astellas Pharma Europe BV, Translational and Development Pharmacology Department, P.O. Box 108, 2350 Leiderdorp, The Netherlands

ARTICLE INFO

Article history:
Received 14 August 2012
Received in revised form
9 November 2012
Accepted 23 November 2012
Available online 12 December 2012

Keywords:
β-Adrenoceptor
Benign prostatic hyperplasia
Lower urinary tract symptom
Mirabegron
Overactive bladder syndrome

ABSTRACT

β_3-Adrenoceptors are major players in detrusor relaxation and have been suggested as a new putative target for the treatment of overactive bladder syndrome. We determined the effects of mirabegron (YM178), a novel β_3-adrenoceptor agonist, on carbachol-induced tone in isolated human detrusor preparations from patients with bladder outflow obstruction (BOO) with and without detrusor overactivity (DO), and from patients with normal bladder function. We compared the effects to those of isoprenaline, a non-selective β-adrenoceptor agonist. Detrusor specimens were obtained from patients with benign prostatic hyperplasia undergoing cystoscopy and from patients undergoing radical prostatectomy/cystectomy (in total 33 donors). Detrusor contractility was evaluated by organ bath studies and strips were incubated with carbachol (1 μM) to induce and enhance tension. Both mirabegron and isoprenaline reduced carbachol-induced tone in tissues from all groups. Isoprenaline decreased tension with higher potency than mirabegron in normal, BOO and BOO+DO detrusor strips with pIC_{50} values of 7.49 ± 0.16 *vs.* 6.23 ± 0.26 ($P=0.0002$), 6.89 ± 0.34 *vs.* 6.04 ± 0.31 ($P=0.01$), and 6.57 ± 0.20 *vs.* 5.41 ± 0.08 ($P<0.0001$, $n=4$), respectively. The maximal relaxant effect of isoprenaline and mirabegron in the normal, BOO and BOO+DO detrusor was $37.7 \pm 14.4\%$ and $36.1 \pm 23.3\%$, $14.4 \pm 12.2\%$ *vs.* $33.4 \pm 21.0\%$ and $18.3 \pm 10.0\%$ *vs.* $28.3 \pm 12.2\%$ ($n=4$, $P>0.05$), respectively. Mirabegron and isoprenaline reduced carbachol-induced tone in both normal bladders and obstructed bladder with and without DO. Isoprenaline had higher potency than mirabegron, but the efficacy of mirabegron effect was the same as that of isoprenaline.

1. Introduction

In animal models, both non-selective and selective β_3-adrenoceptor agonists have been reported to increase bladder capacity with no change in micturition pressure or residual volume (Fujimura et al., 1999; Woods et al., 2001; Kaidoh et al., 2002; Takeda et al., 2002; Nomiya and Yamaguchi, 2003; Takasu et al., 2007). Functional experiments on isolated detrusor smooth muscle from both normal and neurogenic human bladders suggest that the relaxation induced by adrenergic stimulation is mediated mainly through β_3-adrenoceptor activation (Nomiya and Yamaguchi, 2003; Igawa et al., 1999; Igawa et al., 2001). This is supported by the observation that β_3-adrenoceptors account for 97% of the total amount of β-adrenoceptor mRNA in the human urinary bladder (Nomiya and Yamaguchi, 2003). On the other hand, the receptor protein was not determined in this study, and a contribution of other β-adrenoceptors cannot be excluded (Yamaguchi and Chapple, 2007). Nevertheless, the β_3-adrenoceptors appear to be an important therapeutic target, and results from recent clinical studies showed that mirabegron is effective in treating the symptoms of the overactive bladder syndrome (Chapple et al., 2008b; Khullar et al., 2011).

Mirabegron (YM178) has been shown to be more than 446 times as selective for human β_3-adrenoceptors as for β_2-adrenoceptors and β_1-adrenoceptors expressed in Chinese hamster ovary cells (Takasu et al., 2007). Furthermore, mirabegron has been used in organ bath studies showing that the drug relaxed

* Corresponding author. Tel.: +45 47 32 55 01; fax: +45 56 63 38 50.
E-mail addresses: jusj@regionsjaelland.dk (J. Svalø), jrno@heh.regionh.dk (J. Nordling), kirsbouc@rm.dk (K. Bouchelouche), Karl-Erik-Andersson@med.lu.se (K.-E. Andersson), Cees.Korstanje@astellas.com (C. Korstanje), pnb@regionsjaelland.dk (P. Bouchelouche).

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http://dx.doi.org/10.1016/j.ejphar.2012.11.060

normal human urinary bladder strips, being one third as potent as isoprenaline (Takasu et al., 2007).

Taken together, the above results suggest β_3-adrenoceptors as potentially useful targets for drugs intended for the treatment of overactive bladder, including storage symptoms secondary to bladder outflow obstruction (Ursino et al., 2009; Igawa et al., 2010). To the best of our knowledge, there are no published studies comparing the effects of mirabegron on isolated human detrusor muscle from normal and dysfunctional bladders. Therefore, in this pilot study, we studied the effect of mirabegron on tone induced by muscarinic receptor stimulation in patients with normal bladder function and in patients with urodynamically proven bladder outflow obstruction (BOO), with and without detrusor overactivity (DO).

2. Materials and methods

2.1. Patients

Detrusor tissue was obtained from male patients with normal bladder function undergoing radical prostatectomy or cystectomy because of malignancy (ten patients, mean age of 66 years, range 53–77 years). Detrusor specimens were obtained during surgery with special care taken to remove the samples from a non-tumor infiltrating area. Detrusor specimens from BOO patients were obtained in those due to benign prostatic hyperplasia undergoing transurethral resection of the prostate (14 patients, mean age of 68 years, range 57–84 years) or in patients with benign prostatic hyperplasia with known detrusor overactivity (BOO+DO) undergoing cystoscopy (nine patients, mean age of 72 years, range 63–86 years). All BOO patients were urodynamically characterized.

Detrusor specimens were kept in MEM-d-val medium (Invitrogen, UK) at 4 °C and transported immediately to the Smooth Muscle Research Center at Koege Hospital for organ bath studies. The study was approved by the Copenhagen County Ethics Committee.

2.2. Preparation of human detrusor strips

Upon arrival the tissue was immediately placed in cold Tyrode's solution (composition in mM: NaCl 137, KCl 3, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.4, $NaHCO_3$ 12, glucose 6, pH 7.4) (Sigma-Aldrich, Denmark). Using a stereo microscope the detrusor muscle was separated from the mucosa and adventitia, and cut into pieces of approximately $2 \times 4\ mm^2$. The strips were mounted between 2 stainless steel pins connected to a force transducer at a resting tension of 3 to 4 mN. The tissues were allowed to equilibrate for 1 h in 1 ml Tyrode's solution heated to and maintained at 37 °C and continuously gassed with 95% O_2 and 5% CO_2. The preparation was subjected to fresh bath solutions every 20 min during equilibration. The responsiveness of each strip to 80 mM KCl was then recorded and strips that did not respond were discarded. The analog signal output was digitalized (100 Hz sampling rate) with a Powerlab 8 and visualized and analyzed using LabChart 7 software (AD Instruments, UK).

2.2.1. In vitro contraction/relaxation experiments

In the three patient groups, the contractile effects of carbachol (non-subtype selective muscarinic receptor agonist) were examined. Increasing concentrations (from 10 nM to 10 μM) of carbachol were added cumulatively to produce a concentration–response curve. The tonic response to carbachol is presented in mN.

For relaxation experiments strips were taken from all the three patient groups and after the stabilization period, the tissues were pre-contracted with 1 μM carbachol. When the contraction had stabilized (approximately 90 min), increasing concentrations of isoprenaline (non-selective β-adrenoceptor agonist, from 1 nM to 10 μM) or mirabegron (selective β_3-adrenoceptor agonist, 1 nM to 30 μM) were added cumulatively with a time-interval of approximately 10 min (at steady state) to produce concentration–relaxation curves. Tissue contractility (mean tension) was measured using LabChart 7 (AD Instruments, UK). In parallel, experiments were performed to control for time- or vehicle-dependent changes in mean tension. The relaxation responses to β-adrenoceptor agonist were calculated as 100% — percentage of the mean tension after pre-contraction by 1 μM carbachol.

2.3. Drugs

Mirabegron ((R)-2-(2-aminothiazol-4-yl)-4′-{2-[(2-hydroxy-2-phenylethyl)amino]-ethyl} acetanilide) was synthesized by Astellas Pharma Inc. (Tokyo, Japan), dissolved in DMSO and diluted in buffer. All other chemicals were purchased from Sigma-Aldrich (Denmark). Carbachol and KCl were dissolved in buffer and isoprenaline (1(3′,4′Dihydroxyphenyl)-2-isopropyl aminoethanol hydrochloride) was dissolved in DMSO+0.1 mM ascorbic acid. Vehicle controls were composed of DMSO or DMSO+0.1 mM ascorbic acid diluted in buffer for mirabegron and isoprenaline experiments. The final bath concentration of DMSO never exceeded 0.04%.

2.4. Data analysis

From each bladder several strips were prepared and exposed to either isoprenaline/mirabegron or vehicle cumulatively. The response was measured as mean tension within 2 min and an average was calculated for each patient. An average was calculated from "*n*" patients and data are presented as mean values ± S.E.M. Potency (EC_{50}; IC_{50}) and maximum responses (E_{max}) were calculated from non-linear curve fitting (with variable slope) of the concentration–response curves using the GraphPad Prism 5.03 (GraphPad Software, CA, USA). Statistical differences were evaluated using one- or two-way ANOVA followed by Bonferroni post-hoc test or unpaired t-test with a value of $P < 0.05$ considered to be statistically significant. All graphical presentations were prepared with the GraphPad Prism.

3. Results

3.1. Effects of carbachol

Carbachol induced a concentration-dependent increase in tension in all the three patient groups (Fig. 1). Carbachol induced

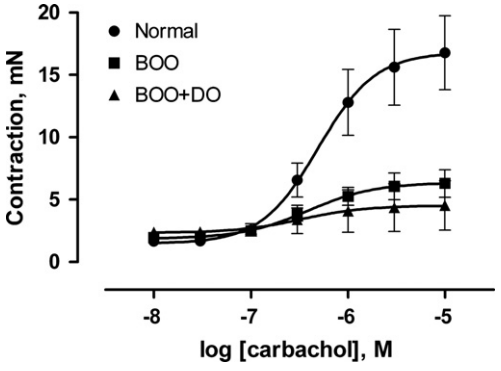

Fig. 1. Carbachol-induced responses of detrusor muscle in normal bladder and outflow obstructed bladder with and without detrusor overactivity. Effect of carbachol (10 nM–10 μM) in normal (circles), BOO (squares) and BOO+DO (triangles) on mean tension in human detrusor strips. Data are presented as mean ± S.E.M. of $n = 7$, 6 and 2 patients.