European Journal of Pharmacology 618 (2009) 76–83

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Pulmonary, Gastrointestinal and Urogenital Pharmacology

Involvement of β_3-adrenoceptors in mouse urinary bladder function: Role in detrusor muscle relaxation and micturition reflex

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ARTICLE INFO

Article history:
Received 2 March 2009
Received in revised form 29 June 2009
Accepted 9 July 2009
Available online 18 July 2009

Keywords:
Urinary bladder
Cystometry
β_3-adrenoceptor
Mouse
Smooth muscle

ABSTRACT

β_3-adrenoceptor activation produces relaxation of human urinary bladder smooth muscle (detrusor). Therefore, β_3-adrenoceptor agonism is being investigated as a new therapeutic strategy for the treatment of overactive bladder. The aim of the current study was to identify the functional presence of β_3-adrenoceptors in mouse isolated urinary bladder using the selective β_3-adrenoceptor agonist CL316,243 and antagonists SR59230A and L748,337. The effects of CL316,243 on basal tone, spontaneous activity and electrical field stimulation (EFS)-induced contractions were investigated using *in vitro* techniques, while the *in vivo* effects of intravenously administered CL316,243 on the micturition reflex were investigated using cystometry. CL316,243 decreased basal tone ($pEC_{50} = 6.4 \pm 0.4$) as well as spontaneous activity ($53 \pm 7\%$ at 3 μM) and inhibited EFS-induced contractions ($pEC_{50} = 7.0 \pm 0.2$) of the detrusor muscle. The β_3-adrenoceptor antagonist SR59230A (1 μM) significantly inhibited the relaxing effects of CL316,243 on basal tone and neurogenic contractions ($pA_2 = 7.0$ and 7.2, respectively). Another β_3-adrenoceptor antagonist L748,337 (1-10 μM) significantly blocked the CL316,243-evoked inhibition of neurogenic contractions in a concentration-dependent manner ($pK_B = 6.8$), while the selective β_2-adrenoceptor antagonist ICI118,551(30 nM) had no effect. In anesthetized mice, CL316,243 (0.03 and 0.1 mg/kg, i.v.) significantly increased bladder capacity and threshold pressure without a modification of bladder compliance. Moreover, it induced a significant decrease in the amplitude of both micturition and non-voiding contractions. Based on the current results obtained using the β_3-adrenoceptor agonist CL316,243 (as well as various β-adrenoceptor antagonists), functional β_3-adrenoceptors appear to be present in mouse urinary bladder.

1. Introduction

Overactive bladder is defined as urgency, with or without urge incontinence, usually with frequency and nocturia (Abrams et al., 2002). This condition markedly impairs the quality of life of sufferers (Hashim and Abrams, 2007). To date, antimuscarinics represent the most used pharmacological therapy for overactive bladder but their use is associated with mechanistic adverse effects such as dry mouth, constipation, and voiding difficulties (Muhlstein and Deval, 2008). Consequently, overall patient compliance is approximately 30%, indicating a need for alternative therapies.

The urinary bladder is innervated by both the sympathetic and parasympathetic nervous systems. Stimulation of sympathetic nerves contributes to urine storage by relaxing the detrusor muscle through activation of β-adrenoceptors (Andersson and Arner, 2004). These receptors are currently classified into β_1-, β_2- and β_3-adrenoceptor subtypes (Bylund et al., 1994). The role of these three subtypes in bladder relaxation differs between species (Michel and Vrydag, 2006). In human detrusor muscle, relaxation is mainly mediated through β_3-adrenoceptor activation (Igawa et al., 1999; Biers et al., 2006). Thus, the use of β_3-adrenoceptor agonists could be a new therapeutic approach for the treatment of overactive bladder. The potential use of these drugs has been supported by some studies in animals, specifically in rats and dogs. In these species the involvement of β_3-adrenoceptors in bladder relaxation has been demonstrated (Yamazaki et al., 1998). Moreover, administration of β_3-adrenoceptor agonists *in vivo* inhibited acetic acid-induced bladder hyperreflexia in anesthetized rats (Woods et al., 2001) and dogs (Hicks et al., 2007) and also detrusor instability in obstructed conscious rats (Woods et al., 2001).

A recently published paper characterized the β-adrenoceptor subtype responsible for urinary bladder relaxation in the mouse (Wuest et al., 2009). This study concluded that despite the expression of all three β-adrenoceptor subtypes at the mRNA level, detrusor muscle relaxation occurred exclusively *via* β_2-adrenoceptor activation. In contrast, preliminary studies both *in vitro* (Deba et al., 2008) and *in vivo* (Lluel et al., 2007) in our laboratory have suggested the additional involvement of β_3-adrenoceptors in mouse urinary bladder relaxation.

The aim of the current study was to elucidate the role of the β_3-adrenoceptor in mouse detrusor muscle relaxation by investigating the effects of the selective β_3-adrenoceptor agonist, CL316,243 (Bloom

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doi:10.1016/j.ejphar.2009.07.012

et al., 1992) as well as two antagonists, namely SR59230A (Manara et al. 1996) and L748,337 (Candelore et al., 1999). The effects of β_3-adrenoceptor activation were studied on basal tone, spontaneous activity and EFS-induced contractions of isolated urinary bladder. The *in vivo* effects of CL316,243 on the micturition reflex in anesthetized animals were also evaluated using cystometric measurements.

2. Materials and methods

This study was conducted according to European Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

2.1. Animals

Female C57Bl/6J mice aged 11 weeks (18-23 g) were obtained from Janvier Laboratories (Le Genest Saint Lisles, France). All mice were group-housed in cages at least 4 days before the experiments with a free access to food and water.

2.2. In vitro experiments

2.2.1. Preparation and equilibration

Mice were sacrificed by cervical dislocation. The whole urinary bladder was isolated, freed from connective and fat tissues, then, the dome and the base were removed. Two strips of detrusor muscle were prepared from each urinary bladder. Longitudinal strips were suspended in 5 ml glass organ baths containing a oxygenated Krebs solution of the following composition (in mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.7, ascorbic acid 1.1 (pH 7.4, gassed with 95% O_2 and 5% CO_2 at 37 °C). Propranolol (1 μM) and prazosin (1 μM) were added to the Krebs solution in order to block β_1/β_2-adrenoceptors and α_1-adrenoceptors, respectively. In one set of experiments, propranolol and prazosin were not added to the Krebs solution to determine if the effects of CL316,243 on EFS were inhibited by propranolol and to test the effect of the β_2-adrenoceptor antagonist ICI118,551. Detrusor muscle responses were measured using isometric transducers (EMKA Technologies, Paris, France) and recorded using a data acquisition system (PowerLab 16s, AD instruments, Sydney, Australia). Tissues were allowed to equilibrate under a resting tension of 0.5 g for 60 min. These experimental conditions were used for all *in vitro* experiments.

2.2.2. Relaxation studies on basal tone and spontaneous activity

Following the 60 min equilibration period, SR59230A (1 μM) or its solvent (distilled water) were incubated for 45 min. Then, cumulative concentration-response curves to CL316,243 (range 0.01 to 10 μM) were obtained for each detrusor muscle strip. CL316,243 was added at 10-15 min intervals in half-log unit concentration increments. After addition of the highest agonist concentration, maximal relaxation was determined by addition of 100 μM papaverine. Time-matched control experiments of basal tone were run in the presence of solvents for CL316,243 (Krebs solution) and for SR59230A (distilled water).

The effects of compounds on spontaneous activity were evaluated using integral of the sample interval, calculated as the sum of the data points minus the lowest value in the selection multiplied by the sample interval, before and after addition of the compound. The sample interval was a 5 min period for SR59230A and a 30 sec period for CL316,243. The results were expressed as % of variation of spontaneous activity before compound incubation.

The relaxing effects to CL316,243 on basal tone in the presence of SR59230A or its solvent were measured as the average tension during a steady-state period (30 s) before and following addition of each concentration of CL316,243. The results were expressed as % of the maximum relaxation obtained with 100 μM papaverine.

2.2.3. Relaxation studies on EFS-induced contractions

After equilibration, detrusor muscle strips were exposed to 80 mM KCl to measure their viability. Strips having a contractile response less than 0.3 g were discarded. Two protocols were performed in a set of different experiments.

2.2.3.1. Frequency-response curves. A further 45 min equilibration period was applied after the contraction to KCl. Then a first frequency-response curve (control curve) to electrical field stimulation (EFS) was constructed for each detrusor muscle strip by measuring the amplitude of EFS-induced contractions at stimulation frequencies of 0.625, 1.25, 2.5, 5, 10 and 20 Hz. The EFS parameters were as follows: 800 mA, pulse duration 0.3 ms, train of pulses 2 s every 1 min, during 5 min. A 5 min interval was applied between each frequency. Subsequently, a 60 min washout period was performed and during the last 10 min, CL316,243 (10 μM) or its solvent were added. Then, a second frequency-response curve to EFS was generated using the same EFS parameters as before. At the end of the experiment, 1 μM tetrodotoxin (TTX) was added to verify the neurogenic origin of the contraction. The contractile response to different EFS frequencies in the presence of CL316,243 or its solvent were expressed as % of variation of the control curve.

2.2.3.2. Concentration-response curves. Following a 45 min re-equilibration period after the contraction to KCl, L748,337 (1 - 10 μM), SR59230A (1 μM), ICI118551 (30 nM) or their respective solvents (DMSO for L748,337 and distilled water for SR59230A and ICI118,551) were added. Detrusor muscle strips were then subjected to EFS using the following parameters: 800 mA, frequency of 2.5 Hz, pulse duration 0.3 ms, train of pulses 2 s every 60 s. Cumulative concentration response curves to CL316,243 (range 0.01 to 100 μM) or its solvent were obtained. CL316,243 was added at 10-15 min intervals in half-log unit concentration increments. At the end of some experiments, 1 μM tetrodotoxin (TTX) was added to verify the neurogenic origin of the contraction. The effects of CL316,243 in the presence of antagonists or their solvents were expressed as % of inhibition of the basal response to EFS taken 5 min before the start of the concentration response curve to CL316,243.

2.2.4. Expression of results and statistical analysis

Data are expressed as mean ± S.E.M. Using mean values, concentration response curves to CL316,243 were fitted by nonlinear regression using the GraphPad Prism version 4.0 software (La Jolla, USA) to obtain the following parameters: Emax (maximum relaxation induced by agonist), pEC_{50} (negative logarithm of agonist concentration which induced 50% of the maximal relaxation) and pK_B (negative logarithm of the antagonist dissociation constant). The pK_B value for L748,337 was determined using nonlinear regression analysis as described by Lew and Angus (1995). Briefly, concentration response curves for CL316,243 in the presence of varying concentrations of L748,337 were plotted against the micromolar concentrations of L748,337 and fitted to the following two equations using nonlinear regression: (1) $pEC_{50} = -\log([\text{antagonist}] + 10^{pK_B}) - \log c$; (2) $pEC_{50} = -\log([\text{antagonist}]^S + 10^{pA_2}) - \log c$. The two equations were compared using the F-test and equation (1) was determined to be the more appropriate equation allowing a pK_B estimate. pA_2 (negative logarithm of antagonist concentration producing a 2-fold shift concentration response curve) for SR59230A was calculated from the mean pEC_{50} values of CL316,243 in the presence and absence of the antagonist using the following equation: $pA_2 = -\log[\text{antagonist}] + \log(\text{dose ratio}-1)$. In study of relaxation of spontaneous activity, the effects evoked by CL316,243 or its solvent were statistically assessed using either one-way or two-way ANOVA with repeated measures followed by Student-Newman Keuls, where appropriate. The first and second frequency-response curves for each group and between groups (CL316,243 and vehicle) were compared using two-way ANOVA with repeated measures. Statistical analyses were performed with