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RESEARCH PAPER

Agonist pharmacology at recombinant α_{1A}- and α_{1L}-adrenoceptors and in lower urinary tract α_1-adrenoceptors

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BACKGROUND AND PURPOSE

Two distinct α_1-adrenoceptor phenotypes (α_{1A} and α_{1L}) have recently been demonstrated to originate from a single α_{1A}-adrenoceptor gene. Here, we examined the agonist profiles of recombinant α_{1A} and α_{1L} phenotypes and of lower urinary tract (LUT) α_1-adrenoceptors.

EXPERIMENTAL APPROACH

A series of drugs (A61603, Ro 115–1240, NS-49 , MK017 and ESR1150) originally developed for stress urinary incontinence (SUI) therapy were used to stimulate recombinant α_{1A}- and α_{1L}-adrenoceptor phenotypes, and their potencies and intrinsic activity estimated from Ca^{2+} responses. Agonist-induced contractions were also examined in LUT tissues of rats and humans and in human mesenteric artery and rat tail artery.

KEY RESULTS

All the drugs were potent agonists of the α_{1A}-adrenoceptor compared with the α_{1L}-adrenoceptor phenotype. Among them, Ro 115–1240 was shown to be an α_{1A}-specific partial agonist that produced partial contractions through α_{1A}-adrenoceptors in rat prostate and tail artery, but not in the other LUT tissues and human mesenteric artery. In contrast, P-come 102 showed full agonist activity at α_{1A}- and α_{1L}-adrenoceptors, but was less selective than noradrenaline for α_{1A}-adrenoceptors. Like noradrenaline, P-come 102 was highly potent at inducing contractions in all of the LUT tissues tested. However, the potency and intrinsic activity of P-come 102 were significantly lower than those of noradrenaline in human mesenteric artery.

CONCLUSIONS AND IMPLICATIONS

The α_{1A}- and α_{1L}-adrenoceptor phenotypes and LUT α_1-adrenoceptors were demonstrated to have distinct agonist profiles. As adrenergic contractions in LUT are predominantly mediated through α_{1L}-adrenoceptors, the development of α_{1L}-selective agonists may provide clinically useful drugs for SUI therapy.

Abbreviations

A61603, *N-(5-[4,5-dihydro-1H-imidazol-2-y]-2-hydroxy-5,6,7,8-tetrahydronaphtalen-1-yl)methanesulfonamide hydrobromide;* BMY7378, (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride; ESR1150, 4-bromo-N3-imidazolidin-2-ylidine-2,N1,N1-trimethyl-benzene-1,3-diamine hydrochloride; LUT, lower urinary tract; MK017, 2-[(5-chloro-3-isopropyl-2-methylphenyl)methyl]-4,5-dihydro-1H-imidazole hydrochloride; NS-49, (R)-(-)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethanesulphonanilide; P-come 102, 2-[(6-dimethylamino-3-isopropyl-2-methyphenyl)methyl]-4,5-dihydro-1H-imidazole hydrochloride; Ro 115-1240 (Dabuzalgron, R450), N-[6-chloro-3-[(4,5-dihydro-1H-imidazol-2yl)methoxy]-2-methylphenyl]methanesulphonamide; RS-17053, *N-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α, α-dimethyl-1 H-indole-3-ethanamine hydrochloride;* SNAP5089, 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate-N-[3-(4,4-diphenylpiperidin-1-yl)propyl]amide methyl ester; SUI, stress urinary incontinence

Introduction

Three distinct genotypes (α_{1A}, α_{1B} and α_{1D}; receptor nomenclature follows that of Alexander *et al.*, 2011) of α_1-adrenoceptors have been identified, and the pharmacological profiles of these recombinant receptors have been verified to be essentially the same as those of the native receptors (Lomasney *et al.*, 1991; Hieble *et al.*, 1995; Graham *et al.*, 1996; Michelotti *et al.*, 2000; Alexander *et al.*, 2011). Prazosin, a prototypical, specific α_1-adrenoceptor antagonist, shows high affinity for α_{1A}, α_{1B}, and α_{1D}-adrenoceptor subtypes (Lomasney *et al.*, 1991; Muramatsu *et al.*, 1995; Ford *et al.*, 1996). With regard to the α_{1A}-adrenoceptor, A61603 and NS-49 were developed as selective agonists (Knepper *et al.*, 1995; Obika *et al.*, 1995), whereas silodosin, 5-methylurapidil, RS-17053 and SNAP5089 have been characterized as selective antagonists (Ford *et al.*, 1996; Murata *et al.*, 1999; Morishima *et al.*, 2007). Of the three classic α_1-adrenoceptor subtypes, the α_{1D}-adrenoceptor shows the highest affinity for catecholamines and is selectively antagonized by low concentrations of BMY7378 (Lomasney *et al.*, 1991; Perez *et al.*, 1991; Hieble *et al.*, 1995). In contrast, agonists/antagonists selective for the α_{1B}-adrenoceptor have not yet been identified.

In addition to these classic α_1-adrenoceptors, a wide variation in antagonist affinity for prazosin has been demonstrated in native α_1-adrenoceptors from different tissues, and the presence of another subtype (designated as α_{1L}-adrenoceptor) has been proposed (Drew, 1985; Flavahan and Vanhoutte, 1986; Muramatsu *et al.*, 1990; Testa *et al.*, 1993; Ford *et al.*, 1996; Argyle and McGrath, 2000; Su *et al.*, 2008). The pharmacological profile of α_{1L}-adrenoceptors is unique and does not match those of classic α_1-adrenoceptor subtypes: it has a low affinity for prazosin, 5-methylurapidil, RS-17053 and BMY7378, but high affinity for silodosin and tamsulosin (Muramatsu *et al.*, 1995; Ford *et al.*, 1996; Testa *et al.*, 1997; Murata *et al.*, 1999; 2000). Recently, the α_{1L}-adrenoceptor was demonstrated to be another phenotype originating from the α_{1A}-adrenoceptor gene, in addition to the classic α_{1A}-adrenoceptor subtypes (Gray *et al.*, 2008; Muramatsu *et al.*, 2008). In the lower urinary tract (LUT: prostate, urinary bladder neck and urethra) of mammals including humans, of the three α_1-adrenoceptor genes α_{1A}-adrenoceptor mRNA is predominantly expressed (Price *et al.*, 1993; Faure *et al.*, 1994; Nishimune *et al.*, 2012), and it has been proposed that adrenergic contractions are induced by activation of either the α_{1A}- or α_{1L}-adrenoceptor phenotype. Although the identity of these functional phenotypes is still contentious (Marshall *et al.*, 1995; Ford *et al.*, 1996; Testa *et al.*, 1997; Van der Graaf *et al.*, 1997; Michel and Vrydag, 2006; Muramatsu *et al.*, 2008; Nishimune *et al.*, 2012). For some time, α_1-adrenoceptor antagonists have been acknowledged to reduce resistance to urinary flow (Andersson, 1993; Ruffolo and Hieble, 1999), and tamsulosin, silodosin, and alfuzosin are currently used to treat urinary problems in male patients with benign prostatic hyperplasia (Andersson, 2002; Michel and Vrydag, 2006). In contrast, α_1-adrenoceptor agonists produce contractions in the urethra and bladder neck, resulting in an increase in urinary resistance. Therefore, α_1-adrenoceptor agonists may be useful clinically to treat women with stress urinary incontinence (SUI; Taki *et al.*, 1999; Andersson and Wein, 2004). As shown in Figure 1, many α_1-adrenoceptor agonists, such as A61603 (Knepper *et al.*, 1995), Ro 115–1240 (Blue *et al.*, 2004; Musselman *et al.*, 2004), NS-49 (Obika *et al.*, 1995), MK017 (Nishimune *et al.*, 2010) and ESR1150 (Matsumaru *et al.*, 2005), have been tested for this purpose, but to date no successful clinical application has been achieved. This may be due to a moderate/poor efficacy of these agonists in the LUT and problems associated with their toxic side effects, which includes their propensity to increase BP. Therefore, we decided to investigate the pharmacology of agonists at α_{1A}- and α_{1L}-adrenoceptor phenotypes and at LUT α_1-adrenoceptors.

Recently, our group identified a cysteine-rich epidermal growth factor-like domain 1α (CRELD1α) as an α_{1A}-adrenoceptor-interacting protein and found that this protein down-regulates the expression of the α_{1A}-adrenoceptor phenotype (Nishimune *et al.*, 2010). By overexpressing this CRELD1α in α_{1A}-adrenoceptor-transfected cells, we obtained a cell line that predominantly expresses the α_{1L}-adrenoceptor phenotype (US patent no. 8173378). In the present study, we compared the agonist profiles of the recombinant α_{1A}- and α_{1L}-adrenoceptors and then examined the effects of a few representative agonists on LUT α_1-adrenoceptors.

Methods

Cell lines expressing α_1-adrenoceptors

CHO cells expressing human α_{1A}, α_{1B} and α_{1D}-adrenoceptors (CHO-α_{1A}, CHO-α_{1B} and CHO-α_{1D} cells, respectively), and co-expressing human α_{1A}- and α_{1L}-adrenoceptors (CHO-α_{1AL}

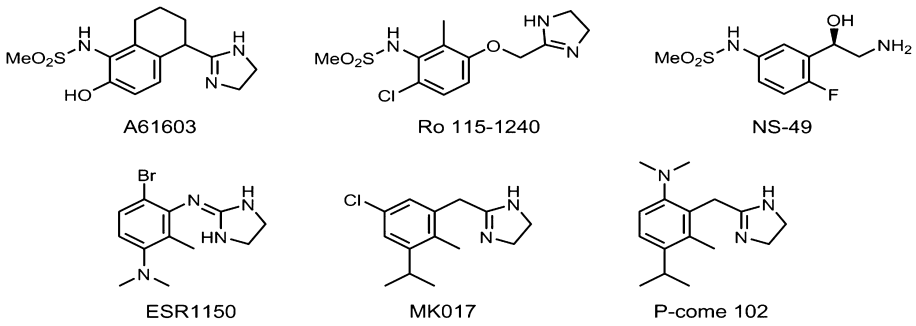

Figure 1

Chemical structures of the representative drugs developed for SUI therapy and tested in the present study. A61603, *N*-(5-[4,5-dihydro-1*H*-imidazol-2-y]-2-hydroxy-5,6,7,8-tetrahydronaphtalen-1-yl)methanesulfonamide hydrobromide; Ro 115–1240 (Dabuzalgron, R450), N-[6-chloro-3-[(4,5-dihydro-1H-imidazol-2yl)methoxy]-2-methylphenyl]methanesulphonamide; NS-49, (R)-(–)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethanesulphonanilide; ESR1150, 4-bromo-*N*3-imidazolidin-2-ylidine-2,*N*1,*N*1-trimethyl-benzene-1,3-diamine hydrochloride; MK017, 2-[(5-chloro-3-isopropyl-2-methylphenyl)methyl]-4,5-dihydro-1*H*-imidazole hydrochloride; P-come 102, 2-[(6-dimethylamino-3-isopropyl-2-methyphenyl)methyl]-4,5-dihydro-1*H*-imidazole hydrochloride.

cells, US patent no. 8173378) were purchased from Pharmacome LCC (Eiheiji-Matsuoka, Fukui, Japan). The densities (fmol·mg^{-1} protein) of each α_1-adrenoceptor expressed were 3000 in CHO-α_{1A} cells, 550 in CHO-α_{1B} cells, 150 in CHO-α_{1D} cells, and 120 (α_{1A}) and 120 (α_{1L}) in CHO-α_{1AL}^{-1} cells. These CHO cells were cultured and maintained as reported previously (Nishimune *et al.*, 2010).

Measurement of the intracellular Ca^{2+} concentration

The intracellular calcium concentration ($[Ca^{2+}]_i$) was measured as described previously (Nishimune *et al.*, 2010). Briefly, CHO cells were loaded with 5 μM Fura-2 AM (Dojindo, Kumamoto, Japan) together with 0.02% pluronic F-127 and 1.4 mM probenecid for 45 min, washed, and then resuspended in Ca^{2+} assay buffer with 1.4 mM probenecid and 3% FBS. The measurements were done at 37°C with Fura-2 ratio fluorometry using a CAF-110 fluorescence spectrophotometer (JASCO, Tokyo, Japan). The cells were exposed to single concentrations of the drugs tested, and the change in $[Ca^{2+}]_i$ was measured for 5 min. At the end of the measurement, the maximum and minimum fluorescence ratios were determined by subsequent addition of Triton X-100 and EGTA respectively. The $[Ca^{2+}]_i$ concentration was calculated using the formula $[Ca^{2+}]_i$ (nM) = $K_d \times [(R - R_{min}) / (R_{max} - R)] \times (S_{f2} / S_{b2})$, where K_d (224 nM at 37°C) is the dissociation constant of Fura-2 for Ca^{2+}; R is the ratio of fluorescence of the sample at 340 nm to that at 380 nm; R_{min} and R_{max} represent the ratios of fluorescence at the same wavelengths in the presence of 0 and saturating Ca^{2+}, respectively; and S_{f2}/S_{b2} is the ratio of fluorescence of Fura-2 at 380 nm in 0 Ca^{2+} to that in saturating Ca^{2+}. In the experiments with CHO-α_{1AL}^{-1} cells, 5 nM prazosin was present throughout the measurement to mask the coexisting α_{1A}-adrenoceptor phenotype and to selectively record the α_{1L} response (Nishimune *et al.*, 2010). The basal level of $[Ca^{2+}]_i$ before stimulation was approximately 100 nM in each cell line, and the net increases in $[Ca^{2+}]_i$ evoked by the drugs tested were normalized to the maximal response to noradrenaline recorded in cells cultured simultaneously.

Rat tissue isolation

Male and female Wistar rats (10–15 weeks of age, Charles River Japan, Inc., Yokohama, Japan) were used in the present study, which was conducted according to the Guidelines for Animal Experiments of the University of Fukui (accredited by the Ministry of Education, Culture, Sports, Science and Technology, Japan). Rats were anaesthetized with isoflurane and killed by cervical dislocation. The prostate, urinary bladder, urethra, and tail artery were rapidly isolated and immersed in modified Krebs-Henseleit solution containing (in mM): NaCl, 112.0; KCl, 5.4; $MgCl_2$, 1.2; $CaCl_2$, 2.0; NaH_2PO_4, 1.2; $NaHCO_3$, 25.5; and D-(+)-glucose, 11.5 (pH 7.4). The solution was oxygenated with a mixture of 95% O_2 and 5% CO_2 and was kept on ice. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Human samples

The study with human samples was performed after obtaining full informed consent according to the guidelines of the Ethics Committee of the University of Fukui. Human urinary bladders were obtained from four male and three female patients (age range: 53–77 years) with urinary bladder cancer. Human mesenteric arteries were obtained from six male and three female patients (age range: 49–75 years) with colon cancer.

Functional studies with isolated tissues

Rat urethra and urinary bladder necks and human urinary bladder necks were transversely cut. Rat prostate was separated into lobes. Human mesenteric arteries (0.5–0.8 mm outer diameter) and rat tail arteries (0.4–0.8 mm outer diameter) were cut into 2 mm long ring preparations. The strips or ring preparations were placed in organ baths containing modified Krebs-Henseleit solution at 37°C. After equilibration for 2 h, noradrenaline was cumulatively applied twice with a 2 h interval, and the isometric tension changes were recorded through a force displacement transducer. Two hours after

recording the second response to noradrenaline in each strip, the preparation was exposed to the test drugs, and the evoked response was normalized to the maximum contraction produced upon the second exposure to noradrenaline in the same preparation. In parallel with the test drug, noradrenaline was applied to other preparations and the third response to noradrenaline was used as a time control. Desipramine (0.3 μM), deoxycorticosterone acetate (5 μM) and propranolol (1 μM) were added to inhibit neural and extraneural uptake of noradrenaline, and to block β-adrenoceptors. In the experiments with human mesenteric arteries and rat tail arteries, N^{ω}-nitro-L-arginine methyl ester (100 μM) was also added to inhibit nitric oxide release from endothelial cells. In the experiments with rat tail arteries, urinary bladders and urethras, rauwolscine (0.1 μM), was added to block α_2-adrenoceptors (Lachnit *et al.*, 1997; I. Muramatsu, unpubl. obs.). All of these blockers were added for at least 40 min before and during every contractile response. To identify the α_1-adrenoceptor subtype, prazosin (1 or 10 nM), silodosin (0.3–3 nM) or BMY7378 (10–20 nM) was added 40 min before and during the third response to noradrenaline or Ro 115–1240, and the responses in the presence of antagonist were compared with the time control that was evoked simultaneously in the absence of antagonists.

Data analysis

The $[Ca^{2+}]_i$ responses to the test drugs were normalized to the maximal response to noradrenaline (a standard drug) obtained from cells cultured at the same time, and the maximal effect of the test drug was expressed as the intrinsic activity. The potency (pEC_{50}) of each drug was estimated from the concentration–response curve, and the relative potency of each test drug was calculated as the ratio between pEC_{50} values for the test drug and noradrenaline. The contractile response to each test drug in the isolated tissue strips was also normalized to the maximal contraction evoked by the second application of noradrenaline in the same strip, and the potency (pEC_{50}) and intrinsic activity were calculated. The antagonist affinity (pK_B) was determined for a single concentration of the antagonist using the concentration-ratio method (Furchgott, 1972). In this case, the concentration-ratio for noradrenaline was generally measured at 50% of the second response to noradrenaline in each preparation, whereas the ratio for Ro 115–1240 was measured at 30% of the second response to noradrenaline (Figure 4C and 4F). The procedure proposed by van Rossum was also applied to insurmountable antagonism (van Rossum, 1963). Data are presented as means ± SEM of the number of experiments (n) and were statistically analysed using Student's *t*-test.

Reagents

The following drugs were used: prazosin hydrochloride, A61603, BMY 7378, 5-methylurapidil and rauwolscine hydrochloride (Sigma-Aldrich Co., St. Louis, MO, USA); L-noradrenaline bitartrate and phenylephrine hydrochloride (Nacalai Tesque, Kyoto, Japan); silodosin (Kissei Pharmaceutical Co., Ltd, Matsumoto, Japan); and tamsulosin (Astellas Pharmaceutical Co., Ltd, Tokyo, Japan). Silodosin (1 mM in DMSO) was stored at –20°C and diluted with DMSO before use. NS-49, Ro 115–1240, P-come 102, MK017 and ESR1150 were synthesized by our group.

Results

Effects of various drugs on recombinant α_1-adrenoceptors

CHO cells expressing different α_1-adrenoceptors were stimulated with various drugs, and the changes in $[Ca^{2+}]_i$ were monitored. Figure 2 shows the representative concentration–response curves for noradrenaline, Ro 115–1240 and P-come 102 in CHO-α_{1A} cells and CHO-α_{1AL}^{-1} cells (in the presence of 5 nM prazosin) in which α_{1A}- or α_{1L}-adrenoceptors were stimulated selectively (Nishimune *et al.*, 2010). Noradrenaline evoked concentration-dependent increases in $[Ca^{2+}]_i$ in both cell lines. The peak levels of $[Ca^{2+}]_i$ evoked by 100 μM noradrenaline were approximately 950 nM in CHO-α_{1A} cells and 550 nM in CHO-α_{1AL} cells (in the presence of 5 nM prazosin), and the pEC_{50} values were 8.3 and 6.0 for the α_{1A}

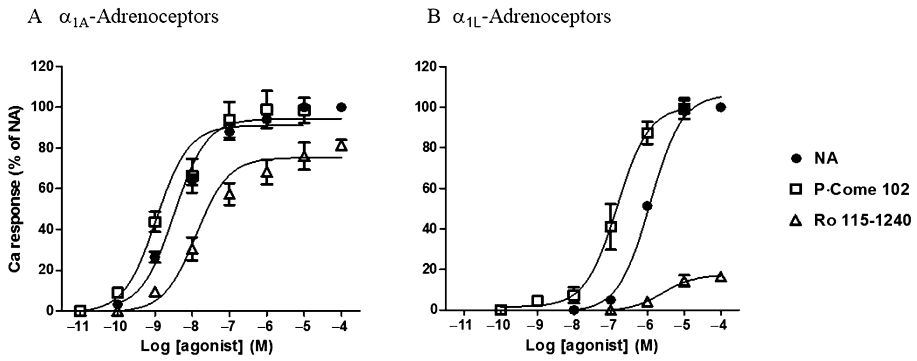

Figure 2

Concentration–response curves for noradrenaline (NA), Ro 115–1240, and P-come 102 at α_{1A}- (A) and α_{1L}-adrenoceptors (B). The increase in intracellular calcium concentration evoked by the various concentrations of agonists was normalized to the maximal response to noradrenaline in the same cells. In (B), the responses mediated through α_{1L}-adrenoceptors were obtained after masking the concomitant α_{1A}-adrenoceptors in the presence of 5 nM prazosin (see Methods). Mean ± SEM of four to six experiments.

and α_{1L} responses respectively (Table 1). The differences in potency and efficacy for noradrenaline were considered to reflect the different densities of α_{1A}- and α_{1L}-adrenoceptor phenotypes expressed in these two cell lines. Ro 115–1240 also activated α_{1A}-adrenoceptors, but the pEC_{50} value and the maximal response were slightly lower than those of noradrenaline (Figure 2A). In contrast, Ro 115–1240 only weakly activated the α_{1L}-adrenoceptors (Figure 2B). Therefore, Ro 115–1240 appears to be a partial agonist that is selective for α_{1A}-adrenoceptors. P-come 102, a newly synthesized compound, fully activated both α_{1A}- and α_{1L}-adrenoceptors.

Noradrenaline and phenylephrine also produced concentration-dependent increases in $[Ca^{2+}]_i$ in CHO-α_{1B} and CHO-α_{1D} cells. The peak level of $[Ca^{2+}]_i$ evoked by 100 μM noradrenaline was approximately 400 nM. The affinities (pEC_{50}) and maximal responses of various drugs at four recombinant α_1-adrenoceptors are summarized in Table 1. Apart from noradrenaline and phenylephrine, all of the agonists tested (P-come 102, A61603, Ro 115–1240, NS-49, MK017 and ESR1150) were essentially inactive at α_{1B}- and α_{1D}-adrenoceptors. As the densities of α_{1A}- and α_{1L}-adrenoceptors expressed in each cell line differed (see Methods), the pEC_{50} values for the various drugs were normalized to that for noradrenaline in the same cell line. Then, the potencies for noradrenaline were assumed to be the same between α_{1A}- and α_{1L}-adrenoceptor phenotypes, and the phenotype selectivity (α_{1L}/α_{1A}) of each drug was estimated (Table 1). In general, large differences in selectivity were not observed between α_{1A}- and α_{1L}-adrenoceptor phenotypes. However, A61603, MK017 and ESR1150 showed higher potency than noradrenaline in both cell lines and showed a trend towards slightly higher selectivity for α_{1A}-adrenoceptors than for α_{1L}-adrenoceptors. In contrast, P-come 102 was less selective than noradrenaline for α_{1A}-adrenoceptors (Table 1). NS-49 was less potent than noradrenaline at α_{1A}- and α_{1L}-adrenoceptors, and its intrinsic activity was lower at α_{1L}-adrenoceptors (0.54) than at α_{1A}-adrenoceptors (0.81).

Effects of noradrenaline, Ro 115–1240 and P-come 102 on rat LUT α_1-adrenoceptors

As we observed distinct agonist profiles between recombinant α_{1A}- and α_{1L}-adrenoceptor phenotypes, we next examined the effects of representative drugs on LUT α_1-adrenoceptors. Here, we used noradrenaline as a standard drug, Ro 115–1240 as an α_{1A}-specific partial agonist, and P-come 102 as an α_{1A}/α_{1L} full agonist with less selectivity for α_{1A}-adrenoceptors. Figure 3A and 3C show the concentration–contraction curves for these three drugs in male and female rat urethras. P-come 102 as well as noradrenaline showed full agonist activity, and the potency of P-come 102 was the same as or slightly higher than that of noradrenaline in both male and female rats (Table 2). In contrast, Ro 115–1240 did not induce a contraction in either male or female urethras. In the urinary bladder neck, full agonist activity was also observed in the responses to noradrenaline and P-come 102 irrespective of sex. These agonist profiles are summarized in Table 2.

The concentration–response curves for noradrenaline in the urethra and urinary bladder neck isolated from male and female rats were slightly inhibited by 10 nM prazosin, with estimated pK_B values of 8.0–8.6 (Figure 3B and 3D for urethra, Table 3). However, BMY7378 (20 nM) had no inhibitory

Table 1
Agonist profiles of various drugs at recombinant α_1-adrenoceptors

Agonist	α_{1A}-adrenoceptor		α_{1L}-adrenoceptor			α_{1B}-adrenoceptor		α_{1D}-adrenoceptor	
	pEC_{50} (RP)	IA	pEC_{50} (RP)	IA	Selectivity (α_{1L}/α_{1A})	pEC_{50} (RP)	IA	pEC_{50} (RP)	IA
Noradrenaline	8.3 ± 0.07 (1.0)	1.00	6.0 ± 0.06 (1.0)	1.00	1.0	8.0 ± 0.18	1.00	8.2 ± 0.11	1.00
Phenylephrine	7.1 ± 0.10 (0.06)	0.88 ± 0.02	5.3 ± 0.11 (0.2)	0.91 ± 0.03	3.2	7.3 ± 0.15 (0.2)	1.07 ± 0.07	6.7 ± 0.24 (0.03)	0.62 ± 0.07
P-come 102	8.6 ± 0.30 (2.0)	1.00 ± 0.10	6.8 ± 0.16 (6.3)	0.98 ± 0.06	3.2		0.08 ± 0.02		0.10 ± 0.05
A61603	10.1 ± 0.19 (63.1)	0.92 ± 0.06	7.3 ± 0.15 (20.0)	1.11 ± 0.08	0.3		0.06 ± 0.00		0.07 ± 0.00
Ro 115–1240	7.7 ± 0.11 (0.3)	0.77 ± 0.02	5.6 ± 0.27 (0.4)	0.17 ± 0.02	1.6		0.00		0.06 ± 0.00
NS-49	7.8 ± 0.11 (0.3)	0.81 ± 0.02	5.5 ± 0.11 (0.3)	0.54 ± 0.02	1.0		0.10 ± 0.03		0.00
MK017	10.3 ± 0.18 (100)	0.73 ± 0.03	7.8 ± 0.11 (63.1)	0.88 ± 0.04	0.6		0.16 ± 0.01		0.14 ± 0.06
ESR1150	9.1 ± 0.08 (6.3)	0.96 ± 0.02	6.5 ± 0.26 (3.2)	0.91 ± 0.07	0.5		0.12 ± 0.01		0.00

RP (relative potency), potency of tested drug was compared with that of noradrenaline at the same α_1-adrenoceptor subtype; IA (intrinsic activity), maximal response to tested drug relative to that of noradrenaline.
Selectivity (α_{1L}/α_{1A}): Selectivity of tested drug at α_{1L}-adrenoceptor against α_{1A}-adrenoceptor, where the affinities for noradrenaline in α_{1L}- and α_{1A}-responses were normalized as equal.
Mean±SEM of three to six experiments.

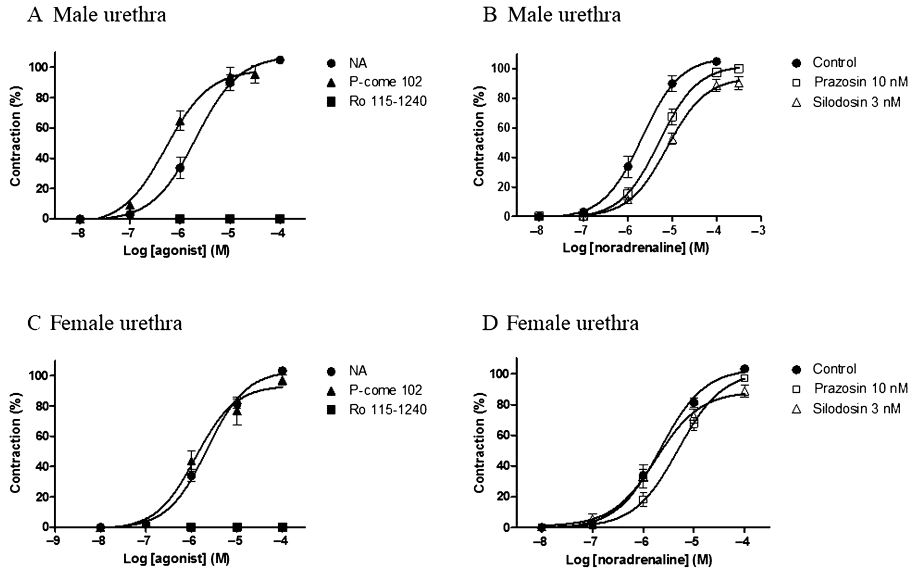

Figure 3

Concentration–response curves for noradrenaline (NA), Ro 115–1240, and P-come 102 in male (A, B) and female (C, D) rat urethras. Maximal contractions induced by a second application of noradrenaline were taken as 100%, and the responses to the third application of noradrenaline or tested agonists were evaluated in the same preparations. (A, C) Concentration-dependent contractions induced by three drugs in male and female rat urethras. (B, D) Effects of 10 nM prazosin and 3 nM silodosin on concentration–response curves for noradrenaline in male and female urethras. Mean ± SEM of four to seven experiments. NA, not applicable.

Table 2

Agonist activities of noradrenaline, P-come 102 and Ro 115–1240 at native α_1-adrenoceptors

			Noradrenaline		P-come 102		Ro 115–1240	
	Tissue		pEC_{50}	IA	pEC_{50}	IA	pEC_{50}	IA
Rat								
	Urethra	Male	5.7 ± 0.09 (5M)	1.05 ± 0.02	6.3 ± 0.11* (5M)	0.96 ± 0.06	– (5M)	0
		Female	5.7 ± 0.06 (5F)	1.03 ± 0.01	5.9 ± 0.13 (4F)	0.97 ± 0.04	– (4F)	0
	Urinary bladder neck	Male	5.9 ± 0.04 (5M)	0.99 ± 0.01	6.2 ± 0.07 (5M)	0.96 ± 0.03	– (4M)	0
		Female	5.7 ± 0.06 (5F)	1.02 ± 0.01	6.2 ± 0.06* (5F)	1.04 ± 0.03	– (4F)	0
	Prostate	Male	7.0 ± 0.07 (8M)	1.0 ± 0.1	7.3 ± 0.07 (7M)	1.0 ± 0.02	6.3 ± 0.09* (8M)	0.60 ± 0.04*
	Tail artery		7.5 ± 0.22 (5M, 5F)	1.01 ± 0.01	6.9 ± 0.11 (4M,4F)	0.99 ± 0.03	7.2 ± 0.13 (5M,5F)	0.55 ± 0.04*
Human								
	Urinary bladder neck		5.4 ± 0.09 (4M,3F)	0.9 ± 0.05	5.9 ± 0.10* (4M,3F)	0.93 ± 0.03	– (4M,3F)	0
	Mesenteric artery		6.4 ± 0.20 (6M,3F)	1.0 ± 0.02	5.7 ± 0.12* (6M,3F)	0.76 ± 0.05*	– (3M,2F)	0

IA (intrinsic activity), maximal contraction induced by tested drug, which was compared with the maximal contraction induced by second application of noradrenaline in the same preparation. The tested response was obtained after twice application of noradrenaline in the same preparation (see Methods). The responses in rat tail artery were obtained in the presence of 10 nM BMY7378. (), number of male (M) and female (F) specimens.

*Significantly different from the value for noradrenaline ($P < 0.05$).

–: no response.

Table 3

Antagonist profiles of the contractile responses to noradrenaline and Ro 115–1240 in various tissues

	Tissue		Agonist	Antagonist (pK_B)		
				Prazosin	Silodosin	BMY7378
Rat						
	Urethra	Male	Noradrenaline	8.2 ± 0.1	9.1 ± 0.0	–
		Female	Noradrenaline	8.0 ± 0.2	–	–
	Urinary bladder neck	Male	Noradrenaline	8.6 ± 0.3	10.3 ± 0.4	–
		Female	Noradrenaline	8.3 ± 0.1	10.0 ± 0.5	–
	Prostate	Male	Noradrenaline	8.2 ± 0.2	9.7 ± 0.3	–
		Male	Ro 115–1240	9.9 ± 0.2	10.3 ± 0.3	–
	Tail artery		Noradrenaline	9.4 ± 0.2 [a]	9.8 ± 0.1[a]	8.3 ± 0.1 [a]
			Ro 115–1240	9.2 ± 0.1 [a]	10.4 ± 0.2[a]	–
Human						
	Urinary bladder neck		Noradrenaline	8.2 ± 0.1	9.7 ± 0.2	–
	Mesenteric artery		Noradrenaline	9.1 ± 0.1	9.1 ± 0.8	–

The pK_B values were estimated at a single concentration of prazosin (10 nM) or silodosin (1 or 3 nM) according to the concentration ratio method (Furchgott, 1972) or van Rossum procedure (van Rossum, 1963). The pK_B values for silodosin in the response to Ro 115–1240 were estimated at 0.3 nM in rat prostate.

[a]The pK_B values for prazosin and silodosin in rat tail artery were obtained in the presence of 10 nM BMY7378, while the pK_B values for BMY7378 were estimated from the ratio at 25% level of control response for noradrenaline (in the absence of BMY7378). The data obtained from both gender were combined in rat tail artery and human urinary bladder neck and mesenteric artery.

–, no inhibition by 20 nM BMY7378 or 3 nM silodosin. Mean ± SEM of four to seven experiments.

effect on the contractile responses to noradrenaline in the urethra and bladder neck. Silodosin (1–3 nM) suppressed the concentration–response curves for noradrenaline in male urethras (Figure 3B) and in male and female bladder necks. However, the contractile responses to noradrenaline in the female urethra were not inhibited by 3 nM silodosin (Figure 3D), suggesting a sex difference in the sensitivity to silodosin of rat urethras.

Next, the contractile responses to these three drugs were examined in the rat prostate. P-come 102 showed full agonist activity with similar potency to noradrenaline. Ro 115–1240 also induced contraction of the prostate, and the intrinsic activity of Ro 115–1240 was 0.60. The contractile response to noradrenaline in the prostate was relatively resistant to 10 nM prazosin, but was inhibited by 1 nM silodosin (Figure 4B). In contrast, the contractions evoked by Ro 115–1240 were noticeably suppressed by both prazosin (1 nM) and silodosin (1 nM; Figure 4C, Table 3).

Effects of noradrenaline, Ro 115–1240, and P-come 102 on α_1-adrenoceptors in rat tail artery

The presence of functional α_{1A}-adrenoceptors has also been reported in rat tail artery (Lachnit *et al.*, 1997; Tanaka *et al.*, 2004), therefore, we examined the effects of all three agonists on these arterial α_1-adrenoceptors. In the presence of 10 nM BMY7378 and 100 nM rauwolscine, to mask α_{1D}-adrenoceptors and α_2-adrenoceptors, respectively, noradrenaline and P-come 102 showed full agonist activity, but Ro 115–1240 was only a partial agonist in the rat tail artery (Figure 4D). In contrast to the simple concentration–response curves for P-come 102 and Ro 115–1240, the curve for noradrenaline, produced by a wide range of noradrenaline concentrations (1 nM–100 μM), was shallow suggesting that distinct α_1-adrenoceptor subtypes (probably α_{1A} and α_{1B} subtypes; Lachnit *et al.*, 1997; Tanaka *et al.*, 2004) are involved in the response to noradrenaline. The concentration–response curves for noradrenaline and Ro 115–1240 were inhibited by 10 nM prazosin and 1 nM silodosin (Figure 4E and 4F, Table 3). We observed no sex differences in the α_1-adrenoceptor responses of the tail artery.

Effects of noradrenaline, Ro 115–1240 and P-come 102 on α_1-adrenoceptors in the urinary bladder neck and mesenteric artery of humans

Finally, we examined the effects of the three drugs on human tissues. Figure 5A and 5C show the concentration–contraction curves for noradrenaline, Ro 115–1240 and P-come 102 in urinary bladder neck and mesenteric artery tissues. P-come 102 showed full agonist activity with slightly higher potency than noradrenaline in the urinary bladder neck preparations (four male and three female patients). However, the potency and intrinsic activity of P-come 102 were lower than that of noradrenaline in the mesenteric artery (six male and three female patients; Table 2). Ro 115–1240 was inactive in both human preparations. The contractile response to noradrenaline in the urinary bladder neck was resistant to 10 nM prazosin, whereas a significant rightward shift of the concentration–contraction relationship was

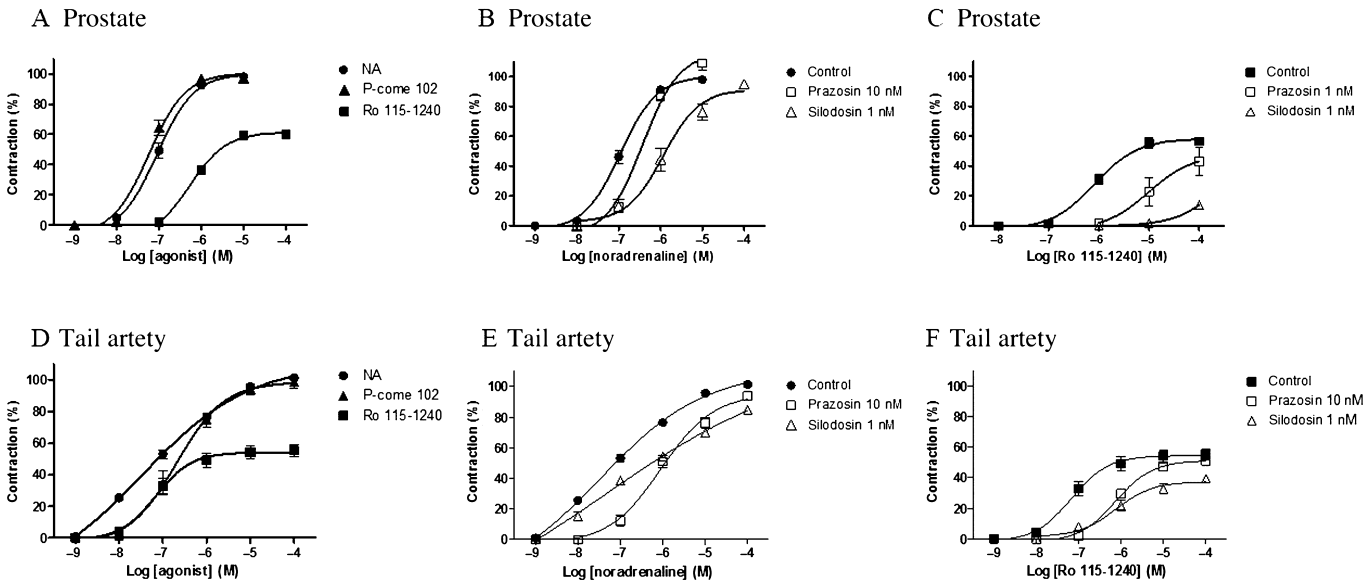

Figure 4

Concentration–response curves for noradrenaline (NA), Ro 115–1240, and P-come 102 in the prostate (A, B, C) and tail artery (D, E, F) of rats. The responses in the tail artery were obtained in the presence of 10 nM BMY7378. (A, D) Concentration-dependent contractions induced by three drugs in the prostate and tail artery. (B, E) Effects of prazosin (10 nM) or silodosin (1 nM) on concentration–response curve for noradrenaline. (C, F) Effects of prazosin (1 or 10 nM) and silodosin (1 nM) on the response to Ro 115–1240. Mean ± SEM of five to seven experiments. NA, not applicable.

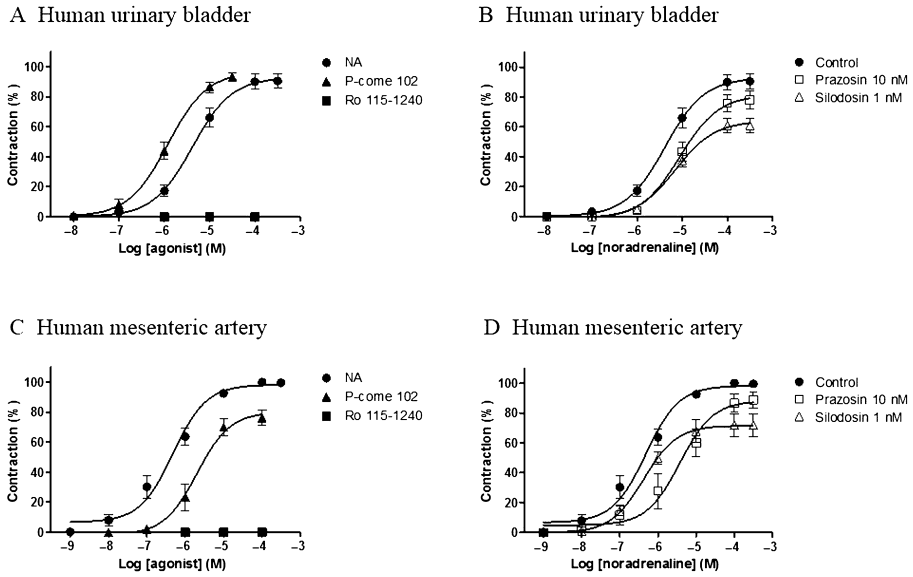

Figure 5

Concentration–response curves for noradrenaline (NA), Ro 115–1240, and P-come 102 in the human urinary bladder (A, B) and mesenteric artery (C, D). (A, C) Concentration-dependent contractions induced by three drugs in the urinary bladder and mesenteric artery. (B, D) Effects of prazosin (10 nM) or silodosin (1 nM) on concentration–response curve for noradrenaline. Mean ± SEM of seven experiments in the urinary bladder and nine in the mesenteric artery. NA, not applicable.

produced by the same concentration of prazosin in the mesenteric artery (Figure 5B and 5D). Silodosin (1 nM) caused insurmountable inhibition of the concentration–response curve for noradrenaline in the urinary bladder neck (Figure 5B) and suppressed the responses to high concentrations of noradrenaline in the mesenteric artery (Figure 5D). BMY7378 (20 nM) failed to affect the contractile response to noradrenaline in either tissue (Table 3).

Discussion

In the LUT it has been proposed that adrenergic contractions are induced by activation of α_1-adrenoceptors, but the identity of its functional phenotype is still contentious. We have hypothesized that this may be due to the expression of distinct α_1-adrenoceptor phenotypes (α_{1A} and α_{1L}) from a single α_{1A}-adrenoceptor gene. Therefore, in the present study, we first examined the agonist profiles of recombinant α_{1A}- and α_{1L}-adrenoceptor phenotypes using various drugs, A61603, NS-49, Ro 115–1240 and ESR1150 (Figure 1), which have been developed for SUI therapy (Knepper *et al.*, 1995; Obika *et al.*, 1995; Blue *et al.*, 2004; Musselman *et al.*, 2004; Matsumaru *et al.*, 2005; Nishimune *et al.*, 2010). Other than noradrenaline and phenylephrine, these drugs showed high specificity for α_{1A}- and α_{1L}-adrenoceptor phenotypes and were essentially inactive at the α_{1B}- and α_{1D}-adrenoceptors. In particular, A61603, MK017 and ESR1150 were more potent than noradrenaline at the α_{1A}-adrenoceptor phenotype. P-come 102 (a newly synthesized compound) was less selective than noradrenaline for the α_{1A} phenotype. With regard to their intrinsic activity, A61603, ESR1150 and P-come 102 showed full-agonist activity at both α_{1A} and α_{1L} phenotypes, whereas NS-49, Ro 115–1240 and MK017 were partial agonists at both phenotypes. From these results, most of the agonists developed to treat SUI appeared to have high activity at the α_{1A}-adrenoceptor phenotype. In particular, Ro 115–1240 and NS-49 appeared to be specific for the α_{1A}-adrenoceptor phenotype, because their intrinsic activity at the α_{1L}-adrenoceptor phenotype was minimal. In contrast to these drugs, P-come 102 was a full agonist at α_{1A}- and α_{1L}-adrenoceptor phenotypes.

We next characterized the pharmacological properties of functional α_1-adrenoceptors in LUT tissues (rat prostate, urethra, and urinary bladder neck, and human urinary bladder neck) using three selected agonists. P-come 102 showed full-agonist activity in the LUT tissues, whereas Ro 115–1240 (a partial agonist of α_{1A}-adrenoceptor) failed to produce a contraction in the LUT tissues tested other than the rat prostate. Pharmacological analysis showed that the contractile responses to noradrenaline in LUT tissues (except female rat urethra) were relatively resistant to inhibition by prazosin (α_{1A}, α_{1B} and α_{1D}-selective antagonist at ~3 nM), highly sensitive to silodosin (α_{1A} and α_{1L}-selective antagonist at ~3 nM), but insensitive to BMY7378 (α_{1D}-selective antagonist at ~20 nM), suggesting the predominant involvement of the α_{1L}-adrenoceptor phenotype in the contractile responses to noradrenaline in these LUT tissues. These results conform with those found previously in human prostate and other LUT tissues of many species (Testa *et al.*, 1993; 1997; Ford *et al.*, 1996; Van der Graaf *et al.*, 1997; Morishima *et al.*, 2007; Nishimune *et al.*, 2012). In human prostate, Ro 115–1240 was also found to be inactive, but P-come 102 as well as noradrenaline produced α_{1L}–adrenoceptor-mediated contractions (I. Muramatsu, unpubl. obs.).

In contrast to the male rat urethra, the contractile response to noradrenaline in female rat urethra was not suppressed by 3 nM silodosin (Figure 3D). This silodosin-resistant contraction was observed in the presence of BMY7378 (α_{1D} antagonist) and rauwolscine (α_2 antagonist) and was inhibited by prazosin with a low pK_B value (8.0). This atypical feature of female urethra α_1-adrenoceptors is not compatible with the criteria of α_1-adrenoceptor classification, and suggests the presence of another phenotype showing a unique drug-binding property. Apart from its low sensitivity to prazosin (pK_B = 8.38; Taki *et al.*, 1999), no detailed information on women urethra α_1-adrenoceptors has been reported. Therefore, further analysis with human materials is urgently needed to determine the pharmacological properties of α_1-adrenoceptors in human urethra.

In contrast to the LUT tissues, the rat prostate and tail artery produced a partial contraction in response to Ro 115–1240, and these contractions were potently inhibited by prazosin and silodosin. α_{1A}–Adrenoceptor-mediated contractions have been reported in rat prostate (Yazawa and Honda, 1993; Forray *et al.*, 1994; Chang *et al.*, 2000) and rat tail artery previously (Lachnit *et al.*, 1997; Tanaka *et al.*, 2004). The present results in rat prostate and tail artery show that Ro 115–1240 can act as a selective agonist on rat α_{1A}-adrenoceptors. As α_{1A}- and α_{1L}-adrenoceptor phenotypes have been demonstrated to occur concomitantly in many tissues (Hiraizumi-Hiraoka *et al.*, 2004; Morishima *et al.*, 2007; 2008; Muramatsu *et al.*, 2008), the inability of Ro 115–1240 to induce a contraction in LUT tissues, other than the rat prostate, may reflect tissue-dependent differences either in the coupling efficiency to the contractile system between the α_{1A}- and α_{1L}-adrenoceptor phenotypes or in the density of expression of each subtype. Although the exact mechanism of these different responses needs to be explored in future studies, the present findings suggest that distinct phenotypes originating from a single receptor gene are independently activated by different agonists.

To reduce the adverse effects associated with SUI treatments, 'uroselectivity' (that is drugs that act selectively on LUT compared with other tissues such as vascular vessels) should be a prerequisite in the development of therapeutic agonists (Andersson and Wein, 2004). Previous studies have reported that human blood vessels are regulated through α_{1A}- and/or α_{1B}-adrenoceptors (Rudner *et al.*, 1999; Michelotti *et al.*, 2000; Murata *et al.*, 2000). The present results with prazosin and silodosin show that α_{1A}- and α_{1B}-adrenoceptors coexist in human mesenteric artery and are both involved in the contractile response to noradrenaline, although the contribution of the α_{1B}-adrenoceptors appears to be more dominant. The lower activity of P-come 102 relative to noradrenaline in human mesenteric artery and the lack of response to Ro 115–1240 may partly reflect a difference in the densities of expression of each α_1-adrenoceptor subtype or the minor involvement of the α_{1L}-adrenoceptor phenotype.

Recent studies have revealed that many GPCRs including α_{1A}-adrenoceptors have the capacity to interact with more than one G-protein subtype as well as induce alternative signalling pathways or effector proteins, resulting in drug-dependent functional selectivity (Audet and Bouvier, 2008; Evans *et al.*, 2010; Kenakin and Miller, 2010). In the present study, we measured $[Ca^{2+}]_i$ and smooth muscle contraction to determine LUT function. However, if different biochemical approaches are applied, distinct agonist profiles or distinct subtype selectivities may be revealed, which may be relevant for the detection of new clinical therapies.

In summary, the present results revealed different agonist profiles between recombinant α_{1A}- and α_{1L}-adrenoceptor

phenotypes and suggest that the α_{1L}-adrenoceptor phenotype is predominantly involved in the contractile responses of LUT. However, we do not yet have selective or specific agonists for the α_{1L}-adrenoceptor phenotype and future studies are needed to develop such compounds that may then provide uroselective and clinically useful drugs for SUI therapy.

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Conflict of interest

The authors have no conflict of interest.

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