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Analysis of α_1-adrenoceptors in rabbit lower urinary tract and mesenteric artery

Pieter H. Van der Graaf, Valérie Deplanne, Chantal Duquenne, Itzchak Angel *

Synthélabo Recherche (L.E.R.S.), Department of Internal Medicine, B.P. 248, 10 rue des Carrières, 92500 Rueil Malmaison, France

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Abstract

In this study, we have investigated the effects of a series of α_1-adrenoceptor antagonists on the phenylephrine-mediated contractions of rabbit isolated prostate, urethra, trigone and mesenteric artery. With the exception of RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride), the antagonists displayed the lowest potency in the urethra. Catecholamine uptake$_1$ and uptake$_2$ appeared not to be the cause for the low pK_B/pA_2 values obtained in the urethra because cocaine and corticosterone had no effect on the potency of phenylephrine in this tissue. The low potencies displayed by prazosin, RS-17053 and HV723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)amino)propyl)benzene-acetonitrile fumarate) suggest that the functional receptors in all four tissues belong to the α_{1L}-adrenoceptor class. Whether or not the significant between-tissue differences in antagonist potencies are due to heterogeneity of this receptor class remains to be elucidated.

Keywords: α_1-Adrenoceptor; Mesenteric artery; Prostate; Trigone; Urethra; (Rabbit)

1. Introduction

Radioligand binding and molecular biology experiments have revealed that there are at least three subtypes of α_1-adrenoceptors, now referred to as α_{1A}, α_{1B} and α_{1D} (Hieble et al., 1995b). Various groups have shown that the α_1-adrenoceptor antagonist, prazosin, does not discriminate between these subtypes (see Ford et al., 1994; Hieble et al., 1995a,b; Michel et al., 1995). Functional pharmacological studies, however, have resulted in a subdivision of α_1-adrenoceptors that is based on the selectivity of prazosin. Digges and Summers (1983) were the first to show that prazosin was more potent as an antagonist of noradrenaline-mediated contractions of rat aorta ($pA_2 = 9.4$) than of portal vein ($pA_2 = 8.4$). Holck et al. (1983) found that in rabbit pulmonary artery prazosin expressed a 10-fold higher potency against clonidine than against methoxamine. Subsequently, several reviews (Agrawal et al., 1984; Medgett and Langer, 1984; Drew, 1985; Flavahan and Vanhoutte, 1986) drew attention to the wide between-tissue variation in affinity values reported in the literature for prazosin and also for yohimbine. Flavahan and Vanhoutte (1986) proposed the existence of two α_1-adrenoceptors, one with high affinity for prazosin and yohimbine and one with low affinity for these two ligands. These two α_1-adrenoceptors were designated α_{1H} and α_{1L}, respectively (see McGrath and Wilson, 1988). More recently, Muramatsu et al. (1990) found that in three dog vascular preparations, yohimbine displayed high affinity and prazosin relatively low affinity, inconsistent with the α_{1H}/α_{1L} scheme. Therefore, they hypothesised the existence of a second α_1-adrenoceptor with low affinity for prazosin, which was designated α_{1N}-adrenoceptor (Muramatsu et al., 1990). In an attempt to reconcile the classification schemes from binding, molecular biological and functional studies the same group proposed to divide further the α_{1H} subtype into α_{1A}, α_{1B} and α_{1C}, thus suggesting the existence of five α_1-adrenoceptor subtypes (Muramatsu et al., 1991). Recently, Ford et al. (1994) have proposed a similar classification with four subtypes: three with high affinity for prazosin (α_{1A}, α_{1B} and α_{1D}) and one subtype which displays low affinity for prazosin (α_{1L}-adrenoceptor). In contrast to the three α_1-adrenoceptor subtypes that are recognised by the Adrenoceptor Nomenclature Committee (Hieble et al., 1995b), members of the α_{1L}-adrenoceptor class have not yet been cloned. Furthermore, the pharmacological profile of the α_{1L}-adrenoceptor is relatively unknown and to date no selective ligands have been reported.

* Corresponding author. Tel. (33-1) 4139-1371; Fax: (33-1) 4139-1305.

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It is well established that postsynaptic α_1-adrenoceptors play an important role in the regulation of smooth-muscle tone in the lower urinary tract (see Andersson, 1993). Interestingly, various groups have reported that prazosin antagonises the contractile responses of rabbit urethra, prostate and trigone to noradrenaline and phenylephrine with associated pA_2/pK_B values less than 8.5 (Honda et al., 1985; Yoshida et al., 1991; Lefèvre-Borg et al., 1993; Testa et al., 1993; Auguet et al., 1995; Hiraoka et al., 1995). This could suggest that the α_1-adrenoceptors in these tissues cannot be classified into the currently accepted scheme with the three (α_{1A}, α_{1B} and α_{1D}) α_1-adrenoceptors which display subnanomolar affinity for prazosin, but rather have the characteristics of the pharmacologically defined α_{1L}-adrenoceptor class. In an attempt to illuminate further the pharmacological characteristics of the putative α_{1L}-adrenoceptors in the lower urinary tract, we have studied the effects of a series of α_1-adrenoceptor antagonists from different chemical classes on the phenylephrine-mediated contractions of rabbit trigone, urethra and prostate. For comparison, the same compounds were also tested in the rabbit isolated mesenteric artery, since it has been proposed that the contraction of this tissue to noradrenaline is mediated by α_{1L}-adrenoceptors (Muramatsu et al., 1990).

2. Materials and methods

2.1. Rabbit isolated smooth muscle preparations

Male New Zealand rabbits (approx. 18 weeks; ESD France) were killed by cervical dislocation and exsanguination and the mesenteric artery, trigone, urethra and prostate (transverse smooth muscles from the posterior central wall) were removed and placed in modified Krebs-Henseleit solution of the following (mM) composition: NaCl 114.0, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.7 and ascorbic acid 1.1. The intimal surface of the mesenteric artery was gently rubbed with a polythene tube to remove the endothelium. Tissues were cleared of surrounding adipose tissue, cut in rings of approx. 3 mm (urethra and mesenteric artery) or strips of approx. 2 cm (prostate and trigone) and mounted between two stainless-steel hooks in 20 ml organ baths containing the modified Krebs-Henseleit solution (continuously gassed with 95% O_2 and 5% CO_2 and thermostatically controlled at 37°C). Tissue responses were measured continuously using Grass FT03 isometric transducers and displayed on a Grass 7D polygraph.

2.2. Experimental protocol

Following application of 1 g resting tension, tissues were allowed to equilibrate for 90 min during which time the organ bath fluid was replaced regularly. Subsequently, tissues were exposed to two consecutive concentrations of noradrenaline (30 μM), separated by 60 min washout periods. In the case of the mesenteric artery, the absence of endothelium was confirmed by the lack of relaxant response to 1 μM acetylcholine added at the plateau of the second noradrenaline contractile response. Following a 60 min washout period, a first phenylephrine concentration–effect ($E/$[A]) curve was obtained by cumulative additions as half-log unit concentration increments. Tissues were then washed for 60 min and incubated for 30 min with antagonist, uptake inhibitor or vehicle before a second phenylephrine $E/$[A] curve was obtained. Preliminary experiments had shown that there were no significant shape or location differences between the first and second phenylephrine $E/$[A] curves in any of the tissues (see Section 3). Only one concentration of antagonist was studied in each tissue. Propranolol (1 μM) was present in all experiments to block β-adrenoceptors.

2.3. Analysis

Individual phenylephrine $E/$[A] curves in the absence and presence of antagonist were fitted using the Allfit program (De Lean et al., 1978) to provide estimates of midpoint location (pEC_{50}, that is $-\log EC_{50}$) and upper asymptote (E_{max}). Effects were expressed as percentage of the E_{max} of the first $E/$[A] curve.

When the minimum criterium for competitive antagonism was satisfied, that is when the antagonist produced a parallel rightward shift of the phenylephrine $E/$[A] curve, Schild plots were constructed and estimates of pA_2 and slope were obtained by linear regression (Arunlakshana and Schild, 1959). When the Schild plot slope parameter was not significantly different from unity, the data were re-fitted with the slope constrained to unity so that the antagonist equilibrium constant could be estimated as $pK_B \pm$ S.E. (see Jenkinson, 1991; Jenkinson et al., 1995). The Schild regressions obtained in the four tissues were compared using one-way analysis of covariance (ANOCOVA; see Kenakin, 1993). When only one concentration of antagonist was studied, individual pA_2 values were estimated assuming a Schild plot slope of unity and then used to calculate a mean $pA_2 \pm$ S.E.M. The mean pA_2 values estimated in the four tissues were compared using one-way analysis of variance (ANOVA). For all statistical comparisons, values of $P < 0.05$ were considered to be significant.

2.4. Drugs

Compounds were obtained from the following sources: phenylephrine hydrochloride (Sigma); BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride), WB-4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and 5-methylurapidil (Research Biochemicals

International); alfuzosin, doxazosin, phentolamine, prazosin, tamsulosin and terazosin (Synthélabo); HV723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)amino)propyl)benz fumarate; a gift from Professor I. Muramatsu, Fukui Medical School, Japan); RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine hydrochloride; a gift from Roche Bioscience, USA).

5-Methylurapidil and RS-17053 were dissolved in DMSO (dimethyl sulfoxide) at the initial concentration of 2×10^{-3} M, and diluted in distilled water. All other drugs were dissolved in distilled water.

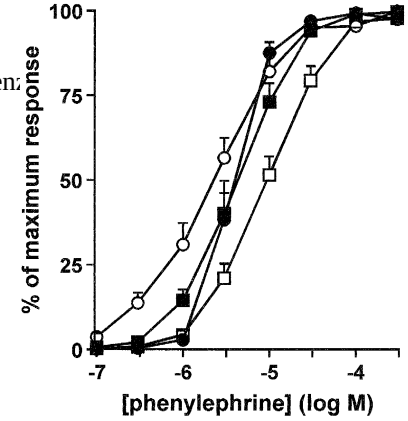

Fig. 1. Concentration–effect curves ($n = 6$–7) for phenylephrine obtained with rabbit isolated mesenteric artery (●), trigone (○), urethra (■) and prostate (□). Error bars indicate S.E.M.

3. Results

3.1. Effects of α_1-adrenoceptor antagonists on phenylephrine-mediated contractions

Phenylephrine (0.1–300 μM) produced concentration-dependent contractions of the mesenteric artery, trigone, urethra and prostate. The associated pEC_{50} values of the first and the second phenylephrine $E/[A]$ curves obtained without antagonist were 5.41 ± 0.08 and 5.22 ± 0.03 ($n = 7$) for mesenteric artery, 5.65 ± 0.11 and 5.57 ± 0.10 ($n = 7$) for trigone, 5.38 ± 0.09 and 5.40 ± 0.06 ($n = 6$) for urethra and 5.02 ± 0.08 and 4.87 ± 0.07 ($n = 7$) for prostate. The first phenylephrine $E/[A]$ curves of each tissue are shown in Fig. 1. Alfuzosin (0.1–10 μM), tamsulosin (3–30 nM), indoramin (0.1–3 μM) and 5-methylurapidil (0.1–1 μM) produced a concentration-dependent, parallel, rightward shift of the phenylephrine $E/[A]$ curves in the four tissues. The data for 5-methylurapidil are shown in Fig. 2 as an example. A Schild analysis was performed and none of the slope parameters was found to be significantly different from unity (0.96 ± 0.12, 1.10 ± 0.22, 0.99 ± 0.12 and 1.18 ± 0.20, respectively, in mesenteric artery, 1.04 ± 0.10, 1.23 ± 0.23, 1.06 ± 0.08 and 0.97 ± 0.13, respectively, in trigone, 0.85 ± 0.12, 1.02 ± 0.15, 0.91 ± 0.13 and 0.77 ± 0.18, respectively, in urethra, and 0.87 ± 0.07, 0.85 ± 0.11, 0.88 ± 0.13 and 1.25 ± 0.21, respectively, in prostate) thus allowing for the estimation of pK_B values (Table 1). In the case of all four antagonists, the lowest pK_B values were obtained in the urethra and comparison of the Schild regressions using ANOCOVA revealed significant between-tissue variation of the pK_B estimates (Table 1).

Phentolamine (1 μM), prazosin (0.1 μM), terazosin (0.3 μM), BMY 7378 (100 μM), RS-17053 (1 μM) and WB-4101 (0.1 μM) were studied at only one concentration. HV723 was studied at 0.03, 0.1, 0.3 and 1 μM in the male rabbit urethra and prostate and at 1 μM in the other tissues (data shown only for 1 μM). With the exception of BMY 7378, these antagonists produced a parallel, rightward shift of the phenylephrine $E/[A]$ curves and pA_2 values were estimated assuming a Schild plot slope parameter of unity (Table 1). As judged by ANOVA, the estimated pA_2 values for phentolamine, prazosin, terazosin and RS-17053 were not identical in the four tissues (Table 1). With the exception of RS-17053, these antagonists also displayed the lowest potency in the urethra, in line with the results obtained with alfuzosin, tamsulosin, indoramin and 5-methylurapidil (see above). BMY 7378 (100 μM) produced significant depression of the upper asymptotes of the phenylephrine $E/[A]$ curves in trigone, urethra and prostate, not consistent with expectations for competitive antagonism (Fig. 3). Notwithstanding this complexity, empirical pA_2 values were estimated from the EC_{50} values of the phenylephrine $E/[A]$ curves in the absence and presence of BMY 7378 as shown in Table 1.

3.2. Investigation of an explanation for the low antagonist potency in the urethra

It is well recognised that the presence of an agonist uptake process can result in underestimation of antagonist affinity (Langer and Trendelenburg, 1969). However, $uptake_1$ and $uptake_2$ appeared not to be the cause for the low pK_B/pA_2 values obtained in the urethra because, as judged by a paired *t*-test, neither cocaine (30 μM) nor corticosterone (30 μM) had a significant effect on the potency of phenylephrine in this tissue ($pEC_{50} = 5.35 \pm 0.09$ and 5.48 ± 0.06 in the absence and presence of cocaine, respectively, $n = 4$, $P > 0.05$; $pEC_{50} = 5.36 \pm 0.08$ and 5.26 ± 0.12 in the absence and presence of corticosterone, respectively, $n = 4$, $P > 0.1$).

3.3. Correlation coefficients between the four tissues investigated, and the cloned α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes

We have studied the correlations (r^2 values) between the pK_B and pA_2 values in rabbit mesenteric artery,

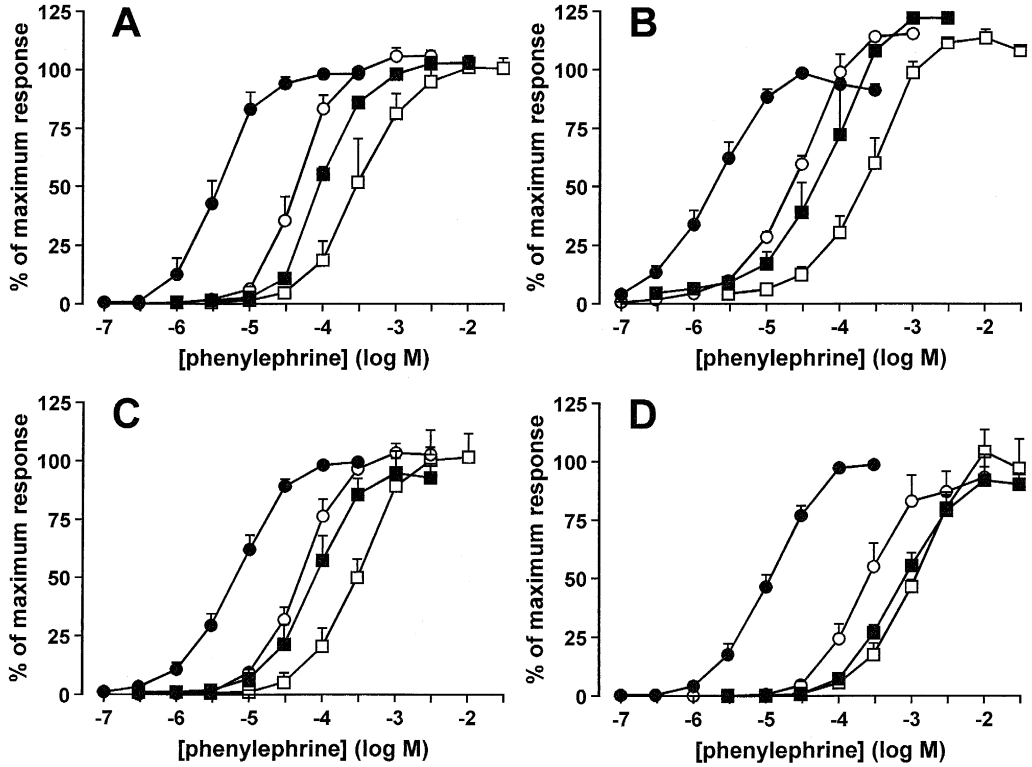

Fig. 2. Concentration–effect curves ($n = 3$–4) for phenylephrine obtained with rabbit isolated (A) mesenteric artery, (B) urethra, (C) trigone and (D) prostate in the absence (●) and presence of 0.1 (○), 0.3 (■) and 1 (□) μM 5-methylurapidil. Error bars indicate S.E.M. Effects are expressed as percentage of the maximum response of the control curve.

trigone, urethra and prostate obtained in the present study (Table 1) and previously reported (Ford et al., 1996) pK_i values for the displacement of [^{3}H]prazosin at bovine α_{1a}-, hamster α_{1b}- and rat α_{1d}-adrenoceptor subtypes. Only those compounds (RS-17053, prazosin, WB-4101, 5-methylurapidil, indoramin, tamsulosin, HV723 and BMY 7378) which were used both by us and by Ford et al. (1996) were included in the analysis.

We did not observe any significant correlation (r^2 values between 0.30 and 0.50) for the four tissues with α_{1b} and α_{1d} subtypes, and for trigone and urethra with the α_{1a} subtype. The only significant correlation was obtained for

Table 1
Results of the analysis of competitive antagonism of phenylephrine-mediated contractions of rabbit isolated mesenteric artery, trigone, urethra and prostate

	Mesenteric artery		Trigone		Urethra		Prostate		Between-tissue comparison [c]
	pK_B or pA_2 [a]	n	pK_B or pA_2 [a]	n	pK_B or pA_2 [a]	n	pK_B or pA_2 [a]	n	
Alfuzosin	7.32 ± 0.06	13	7.22 ± 0.05	13	6.84 ± 0.07	12	7.25 ± 0.04	13	$F(3,46) = 16.3$ ($P < 0.001$)
Indoramin	8.19 ± 0.06	13	8.01 ± 0.04	13	7.56 ± 0.05	10	8.10 ± 0.05	10	$F(3,41) = 23.4$ ($P < 0.001$)
5-Methylurapidil	7.99 ± 0.08	10	8.16 ± 0.05	9	7.70 ± 0.07	10	8.29 ± 0.09	9	$F(3,33) = 11.5$ ($P < 0.001$)
Tamsulosin	9.53 ± 0.09	11	9.26 ± 0.10	12	8.85 ± 0.06	11	9.74 ± 0.05	11	$F(3,40) = 23.8$ ($P < 0.001$)
Phentolamine	8.02 ± 0.05 [a]	3	7.32 ± 0.13 [a]	3	7.20 ± 0.05 [a]	3	7.66 ± 0.06 [a]	3	$F(3,8) = 21.4$ ($P < 0.001$)
Prazosin	8.14 ± 0.03 [a]	3	8.01 ± 0.06 [a]	3	7.71 ± 0.02 [a]	3	8.15 ± 0.13 [a]	4	$F(3,9) = 5.2$ ($P < 0.05$)
Terazosin	7.48 ± 0.03 [a]	3	7.18 ± 0.07 [a]	3	7.10 ± 0.02 [a]	3	7.46 ± 0.09 [a]	3	$F(3,8) = 10.5$ ($P < 0.005$)
BMY 7378	6.18 ± 0.13 [a]	3	(6.11 ± 0.33) [b]	3	(5.73 ± 0.09) [b]	3	(5.92 ± 0.07) [b]	3	$F(3,8) = 1.2$ ($P > 0.3$)
HV723	7.95 ± 0.10 [a]	4	7.74 ± 0.07 [a]	3	7.71 ± 0.0 [a]	4	7.88 ± 0.07 [a]	4	$F(3,11) = 1.9$ ($P > 0.1$)
RS-17053	6.71 ± 0.11 [a]	4	5.67 ± 0.07 [a]	4	5.86 ± 0.14 [a]	4	6.32 ± 0.08 [a]	3	$F(3,11) = 19.9$ ($P < 0.001$)
WB-4101	8.29 ± 0.21 [a]	3	8.20 ± 0.17 [a]	3	7.95 ± 0.03 [a]	3	8.40 ± 0.16 [a]	3	$F(3,8) = 1.5$ ($P > 0.2$)

[a] Estimated pA_2 values obtained by linear regression (see Section 2.3).
[b] Significant reduction of E_{max} in the presence of BMY 7378. Empirical pA_2 values, calculated without prejudice to mechanism, are shown in parentheses.
[c] Analysis of the differences between antagonist potencies in the four tissues was carried out by comparing the mean pA_2 values estimated using one-way analysis of variance (ANOVA).

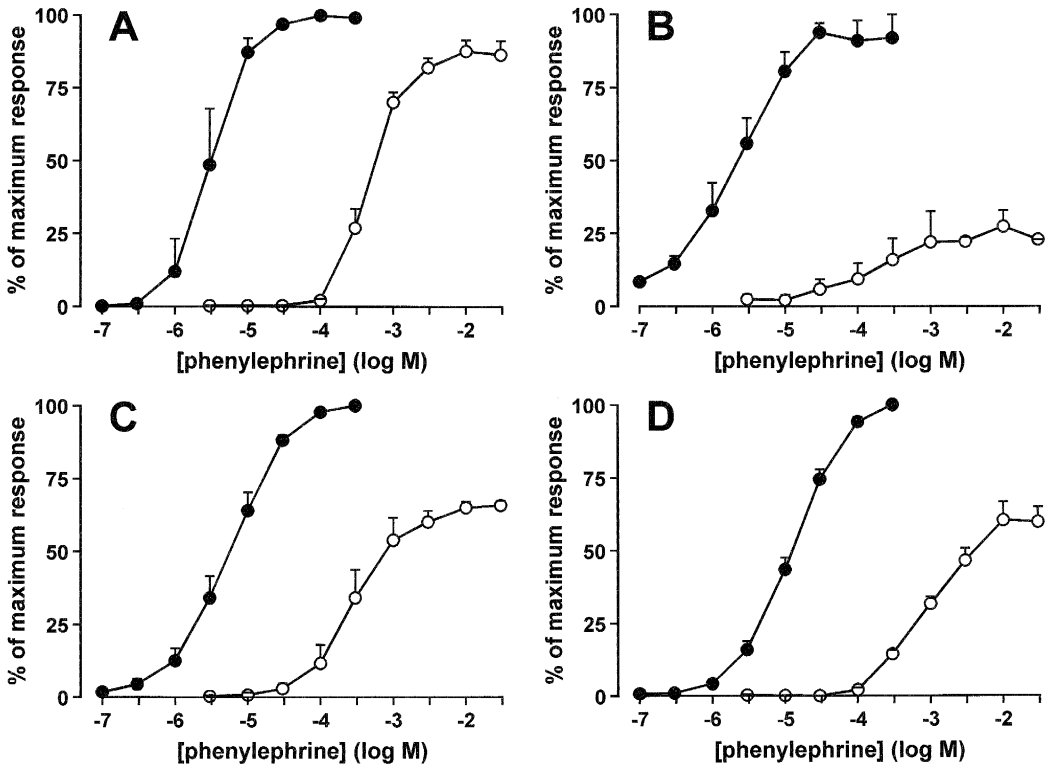

Fig. 3. Concentration–effect curves ($n = 3$) for phenylephrine obtained with rabbit isolated (A) mesenteric artery, (B) urethra, (C) trigone and (D) prostate in the absence (●) and presence (○) of 100 μM BMY 7378. Error bars indicate S.E.M. Effects are expressed as percentage of the maximum response of the control curve.

mesenteric artery and prostate with the α_{1a}-adrenoceptor (0.54 and 0.52, respectively, $P < 0.05$), correlation coefficients which were significant but considerably smaller than the ones obtained from the comparison with the human lower urinary tract (Fig. 4).

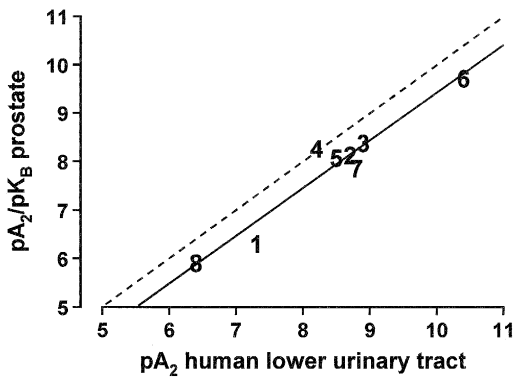

Fig. 4. Relation between pA_2 estimates in human lower urinary tract (Ford et al., 1996) and pK_B/pA_2 estimates (see Table 1) in rabbit prostate for (1) RS-17053, (2) prazosin, (3) WB-4101, (4) 5-methylurapidil, (5) indoramin, (6) tamsulosin, (7) HV723 and (8) BMY 7378. The solid lines were obtained by linear regression, the dashed lines represent the line of identity. High significant correlations were found in the four tissues ($r^2 = 0.96$ ($P < 0.001$) in mesenteric artery; 0.82 ($P < 0.00$) in trigone; 0.91 ($P < 0.00$) in urethra and 0.92 ($P < 0.001$) in prostate).

4. Discussion

It is believed that α_1-adrenoceptors mediating contraction play an important physiological role in the lower urinary tract. Therefore, understanding of the pharmacological characteristics of these receptors may provide the basis for the development of drugs for the treatment of diseases which are associated with dysfunction of the regulation of urinary smooth-muscle tone (e.g., benign prostatic hypertrophy and urinary incontinence; see Andersson, 1988, 1993; Wein, 1991). Recently, Hiraoka et al. (1995) suggested that the contraction of the rabbit prostate to noradrenaline is mediated by the α_{1L}-adrenoceptor. In contrast, it has been proposed that contraction of rabbit urethra is mediated by α_{1A}-adrenoceptors alone (Testa et al., 1993; Auguet et al., 1995) or in combination with α_{1B}-adrenoceptors (Yoshida et al., 1991). In the present study, we obtained low pA_2 values for prazosin in the four tissues studied. These values are in agreement with previous values obtained in our laboratory using five prazosin concentrations (Deplanne and Galzin, 1996) and by others (Honda et al., 1985). On the basis of these low affinity values, we hypothesised that the α_{1L}-adrenoceptor, as defined by Ford et al. (1994), plays and important role not only in rabbit prostate but also in urethra and trigone (see Section 1). This hypothesis was confirmed in the present study, since the potencies displayed by prazosin in the

prostate, urethra and trigone and in the mesenteric artery (previously classified as an 'α_{1L}-adrenoceptor tissue'; Muramatsu et al., 1990) suggest that the affinity of prazosin for the α_1-adrenoceptors in these tissues is more than 10-fold lower than reported affinities for α_{1A}-, α_{1B}- or α_{1D}-adrenoceptor subtypes (see Ford et al., 1994; Hieble et al., 1995a,b; Michel et al., 1995).

BMY 7378, recently described as the first α_{1D}-adrenoceptor-selective antagonist (Goetz et al., 1995), was the only ligand that did not behave in a competitive manner in all assays. We have no explanation for the significant reduction of the maximum contractile response to phenylephrine of urethra, prostate and, most notably, trigone by BMY 7378 (Fig. 3). However, this effect might be related to the relatively high concentration (100 μM) we used in order to obtain a significant rightward shift (preliminary experiments had shown that BMY 7378 at the concentrations of 0.1 and 1 μM did not markedly antagonise the phenylephrine response in any of the tissues, data not shown). This hypothesis is supported by the fact that Goetz et al. (1995), Kenny et al. (1995) and Piascik et al. (1995) did not report noncompetitive antagonism for lower concentrations of BMY 7378 in rat aorta and iliac, caudal, mesenteric and renal arteries. Notwithstanding the noncompetitive behaviour, however, the low potency of BMY 7378 (apparent $pA_2 < 6.2$; pK_i for rat and human cloned α_{1d}-adrenoceptors = 8.2–9.4, Goetz et al., 1995; Kenny et al., 1995; Piascik et al., 1995) indicates that the α_{1D}-adrenoceptor plays no significant role in the contractile responses of the tissues investigated in the present study.

Further evidence for the lack of involvement of receptors resembling any of the three cloned α_1-adrenoceptor subtypes was obtained with RS-17053, since the pA_2 values of 5.7–6.7 obtained with this novel, selective α_{1A}-adrenoceptor antagonist were considerably lower than recently reported affinity values not only for cloned α_{1a}- ($pK_i = 9.5$) but also for α_{1b}- ($pK_i = 7.8$) and α_{1d}-adrenoceptors ($pK_i = 7.8$; Ford et al., 1996). Indeed, when we compared the estimated pK_B and pA_2 values obtained in the present study with recently reported affinities for cloned α_1-adrenoceptor subtypes and pA_2 values obtained in human lower urinary tract (bladder neck, prostate and urethra; Ford et al., 1996), highly significant correlations were found only between the antagonist potencies in the rabbit tissues and the human lower urinary tract (Fig. 4), a tissue which was proposed to contain the putative α_{1L}-adrenoceptor (Ford et al., 1996). In contrast, the comparisons with the cloned receptors yielded either nonsignificant correlations or, in two cases (mesenteric artery and prostate with the α_{1a}-adrenoceptor), correlation coefficients which were significant but considerably smaller than the ones obtained from the comparisons with human lower urinary tract. At first sight, this could suggest that the α_1-adrenoceptors mediating contraction of rabbit mesenteric artery, trigone, urethra and prostate are similar to the putative α_{1L}-adrenoceptor subtype in human lower urinary tract. Interestingly, however, the pA_2 estimates we obtained for RS-17053, most notably in trigone (5.67) and urethra (5.86), were also considerably lower than the value of 7.3 found by Ford et al. (1996) in the human lower urinary tract (Fig. 4). This suggests that the rabbit lower urinary tract may contain functional, prazosin-insensitive, α_1-adrenoceptors which are different from the α_1-adrenoceptors mediating contraction in the human lower urinary tract. This difference might be related to the α_{1L}/α_{1N} classification scheme (see Section 1) proposed by Muramatsu et al. (1990), since HV723, previously described as a selective α_{1N}-adrenoceptor antagonist (Muramatsu et al., 1990; Muramatsu, 1991; Sayet et al., 1993; Kohno et al., 1994), displayed a approx. 10-fold lower potency in our rabbit assays ($pA_2 = 7.7–7.9$) than in the human lower urinary tract ($pA_2 = 8.8$; Ford et al., 1996; Fig. 4).

It appears from reports in the literature that the presence of α_1-adrenoceptors displaying relatively low affinity for prazosin is not restricted to the lower urinary tracts of humans and rabbits. For example, Ohmura et al. (1993) have suggested that the contractile response to noradrenaline in the dog prostate is mediated predominantly through the α_{1L} subtype. Furthermore, Chess-Williams et al. (1994) reported a pK_B value of 8.5 for the antagonism of phenylephrine by prazosin in the rat urethra, which suggests the involvement of an α_{1L}-like adrenoceptor rather than an α_{1A}-adrenoceptor as was proposed by these authors.

At present we do not have an explanation for the significant between-tissue variation in potency which was observed for eight of the eleven antagonists tested (Table 1). The fact that, with the exception of RS-17053, the lowest antagonist potency was always found in the urethra could suggest that the presence of an uptake mechanism for phenylephrine reduces the apparent antagonist affinity for α_1-adrenoceptors in this tissue (see Langer and Trendelenburg, 1969). However, it seems unlikely that uptake$_1$ or uptake$_2$ were the reason for the low antagonist potency in the urethra, since cocaine and corticosterone had no effect on the phenylephrine $E/[A]$ curve. The fact that the low antagonist potency in the urethra was also apparent at concentration ratios even less than 5 indicates that the complexity was not due to the presence of a low-affinity uptake system. Furthermore, other characteristic features associated with the presence of agonist uptake, i.e., flat Schild plots and $E/[A]$ curve steepening in the presence of antagonists (Langer and Trendelenburg, 1969), were not observed by us. Therefore, although agonist uptake cannot be entirely ruled out, the present observations of lower antagonist potencies in the urethra could also suggest that the α_1-adrenoceptor population of this tissue is different from those of the other tissues investigated. This suggestion is different from previous reports by Honda et al. (1985) and Honda and Nakagawa (1986) who concluded that rabbit prostate, trigone and urethra contain a single class of α_1-adrenoceptors. One explanation for these differences could be the higher concentration (10 μM) of

propranolol used by Honda et al. (1985) to block β-adrenoceptors compared with 1 μM in the present study. Another explanation could be related to differences in study design which could differently influence the responses.

In conclusion, in this study we have shown that the α_1-adrenoceptors mediating contraction of rabbit isolated trigone, urethra, prostate and mesenteric artery have pharmacological characteristics which are different from those of the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes. In terms of the α_{1H}-/α_{1L}-/α_{1N}-adrenoceptor classification scheme advocated by Muramatsu et al. (1990), the low potencies of prazosin and HV723 suggest that the functional receptors in all four tissues belong to the α_{1L}-adrenoceptor class. In the case of the mesenteric artery and prostate, this confirms and extends previous studies by Muramatsu et al. (1990) and Hiraoka et al. (1995), respectively. In contrast, in the case of the urethra the present data challenge recent reports which concluded that contraction of this tissue is mediated by α_{1A}-adrenoceptors (Testa et al., 1993; Auguet et al., 1995). Whether or not the significant between-tissue differences in antagonist potencies are due to heterogeneity of the α_{1L}-adrenoceptor class remains to be elucidated.

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