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Native profiles of α_{1A}-adrenoceptor phenotypes in rabbit prostate

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Background and purpose: α_1-Adrenoceptors in the rabbit prostate have been studied because of their controversial pharmacological profiles in functional and radioligand binding studies. The purpose of the present study is to determine the native profiles of α_1-adrenoceptor phenotypes and to clarify their relationship.
Experimental approach: Binding experiments with [^{3}H]-silodosin and [^{3}H]-prazosin were performed using intact tissue segments and crude membrane preparations of rabbit prostate and the results were compared with α_1-adrenoceptor-mediated prostate contraction.
Key results: [^{3}H]-Silodosin at subnanomolar concentrations bound specifically to intact tissue segments of rabbit prostate. However, [^{3}H]-prazosin at the same range of concentrations failed to bind to α_1-adrenoceptors of intact segments. Binding sites of [^{3}H]-silodosin in intact segments were composed of α_{1L} phenotype with low affinities for prazosin (pKi = 7.1), 5-methyurapidil and *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethamine hydrochloride (RS-17053), and α_{1A}-like phenotype with moderate affinity for prazosin (pKi = 8.8) but high affinity for 5-methyurapidil and RS-17053. In contrast, both radioligands bound to a single population of α_1-adrenoceptors in the membrane preparations at the same density with a subnanomolar affinity, showing a typical profile of 'classical' α_{1A}-adrenoceptors (pKi for prazosin = 9.8). The pharmacological profile of α_1-adrenoceptor-mediated prostate contraction was in accord with the α_{1L} phenotype observed by intact segment binding approach.
Conclusions and implications: Three distinct phenotypes (α_{1L} and α_{1A}-like phenotypes in the intact segments and a classical α_{1A} phenotype in the membranes) with different affinities for prazosin were detected in rabbit prostate. It appears that the three phenotypes are phenotypic subtypes of α_{1A}-adrenoceptors, but are not genetically different subtypes.
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Keywords: prostate; receptor phenotype; adrenergic; $\alpha_{1A}$and α_{1L}; radioligand-binding assay

Abbreviations: BMY 7378, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride; B_{max}, maximal binding capacity; RS-17053, *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethamine hydrochloride

Introduction

At present, three distinct subtypes of α_1-adrenoceptors (α_{1A}, α_{1B} and α_{1D}; Alexander *et al* 2008) have been cloned and are known to be widely distributed in mammals including humans (Lomasney *et al*., 1991; Hieble *et al*., 1995; Zhong and Minneman, 1999; Michelotti *et al*., 2000). Pharmacological features of the three classical α_1-adrenoceptors are high (subnanomolar) affinity for prazosin, a prototypic, selective α_1-adrenoceptor antagonist, although several compounds show distinct subtype-selectivity; for example, silodosin, 5-methylurapidil and *N*-[2-(2-cyclopropylmethoxyphenoxy) ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethamine hydrochloride (RS-17053) are selective for α_{1A}-adrenoceptors, and 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5] decane-7,9-dione dihydrochloride (BMY-7378) is selective for α_{1D}-adrenoceptors (Lomasney *et al*., 1991; Hieble *et al*., 1995; Ford *et al*., 1996; Murata *et al*., 1999; Piao *et al*., 2000).

Contraction of prostatic and urethral smooth muscle is mediated by α_1-adrenoceptors, and these receptors are a primary target of α_1-adrenoceptor antagonist therapy of urinary outlet obstruction in patients with benign prostatic hyperplasia (Lepor and Shapiro, 1994; Cooper *et al*., 1999; Chapple, 2001; Andersson, 2002). However, *in vitro*

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functional studies with prostate and some blood vessels have revealed that α_1-adrenoceptor-mediated contractions are relatively insensitive to prazosin, suggesting the presence of a unique α_1-adrenoceptor type, different from the classical α_1-adrenoceptors (Ford *et al*., 1996; Testa *et al*., 1997; Van der Graaf *et al*., 1997; Takeda *et al*., 1999; Argyle and McGrath, 2000; Morishima *et al*., 2007b). It has been proposed that the α_1-adrenoceptors mediating prostate and vascular contractions are of a different subtype from the classical ones; this subtype has been named α_{1L} because of its lower affinity for prazosin (Flavahan and Vanhoutte, 1986; Muramatsu *et al*., 1990). However, a distinct gene corresponding to the putative α_{1L} subtype has not yet been identified; rather, it has been suggested that the α_{1L} subtype may be a functional phenotype of the α_{1A}-adrenoceptor, because the functional studies with recombinant α_{1A}-adrenoceptor have revealed a relatively low affinity for prazosin (Ford *et al*., 1997; Daniels *et al*., 1999). In contrast, binding studies with recombinant α_{1A}-adrenoceptor and with membrane preparations of prostate have revealed a high (subnanomolar) affinity for prazosin, failing to detect the α_{1L} profile (Testa *et al*., 1993; Daniels *et al*., 1999; Piao *et al*., 2000; Ramsay *et al*., 2004). Recently, we demonstrated that α_{1A} and α_{1L} subtypes coexist as distinct entities when radioligand binding studies were performed using the intact segments of several tissues including human prostate but that the α_{1L} subtype converted its profile to α_{1A}-adrenoceptor after homogenization (Hiraizumi-Hiraoka *et al*., 2004; Morishima *et al*., 2007b). More recently, we found that both α_{1A} and α_{1L} subtypes were abolished in α_{1A}-adrenoceptor gene knockout mice (Morishima *et al*., 2007a). These results suggest that for the same α_{1A}-adrenoceptor gene product, different pharmacological phenotypes may be expressed in some native tissues and that this phenotypic diversity may solve the controversy in $\alpha_{1A/L}$ pharmacology (Muramatsu *et al*., 2005; Nelson and Challiss, 2007).

With regard to α_1-adrenoceptors in the prostate, most studies have been performed in rabbits and humans. Because rabbit prostate also expresses α_{1A}-adrenoceptor at the mRNA level (Piao *et al*., 2000), we speculated that rabbit prostate would be an ideal tissue to evaluate different phenotypes derived from the α_{1A}-adrenoceptor gene without contamination by other α_1-adrenoceptors (α_{1B} and α_{1D}). The purpose of this study is to investigate pharmacological phenotypes of α_1-adrenoceptor populations in rabbit prostate and then to explore their relationship. To detect all kinds of phenotypes, binding experiments were conducted in the intact segments and membrane preparations of rabbit prostate using two radioligands: [^{3}H]-silodosin, which has very high apparent affinities for both α_{1A}- and α_{1L}-adrenoceptor subtypes, and [^{3}H]-prazosin, which shows high affinity for α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptor subtypes.

Materials and methods

Animals and tissue isolation

This study was performed according to the Guidelines for Animal Experiments, University of Fukui (which is accredited by the Ministry of Education, Culture, Sports, Science and Technology, Japan). Male pigmented rabbits (1.5–2.5 kg) were anesthetized with sodium pentobarbital (100 mg kg^{-1}) and killed. The prostate was isolated and cleaned in a modified Krebs–Henseleit solution (120.7 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 2.0 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 25.5 mM $NaHCO_3$, and 11.5 mM D-glucose, pH 7.4) aerated with 95% O_2 and 5% CO_2.

Tissue segment binding experiments with [^{3}H]-silodosin and [^{3}H]-prazosin

Tissue segment binding was performed as described previously (Muramatsu *et al*., 2005). Briefly, the prostate isolated from each rabbit was cut into 25–30 pieces (approximately $1.5 \times 3 \times 3$ mm) and used in either a saturation or competition experiment. The segments were incubated with [^{3}H]-silodosin or [^{3}H]-prazosin for 15–16 h at 4 °C in Krebs incubation buffer. Incubation volume was 1 ml, which was enough not to cause radioligand depletion during incubation. The composition of Krebs incubation buffer was essentially the same as a modified Krebs–Henseleit solution, except that the $NaHCO_3$ concentration was reduced to 10.5 mM to adjust the pH to 7.4 in air. [^{3}H]-silodosin or [^{3}H]-prazosin (50–1000 pM) was used in saturation experiments and 300 pM [^{3}H]-silodosin or 500 pM [^{3}H]-prazosin was used for competition experiments. After incubation, the tissue segments were washed with incubation buffer at 4 °C for 1 min and then solubilized in 0.3 M NaOH solution. The non-specific binding was determined in the presence of 30 μM phentolamine. Radioactivity and protein concentrations were determined as described previously (Morishima *et al*., 2008).

Membrane binding experiments with [^{3}H]-silodosin and [^{3}H]-prazosin

Prostates isolated from 3–5 rabbits were pooled and used in one or two saturation or competition experiments. The prostate was homogenized in Krebs incubation buffer containing proteinase inhibitors (Complete, EDTA-free tablet, Roche, Penzberg, Germany). After centrifugation, the crude membrane preparations were used in binding experiments (Morishima *et al*., 2007b, 2008).

Binding saturation and competition experiments with [^{3}H]-silodosin or [^{3}H]-prazosin (50–1000 or 2000 pM) were carried out for 4 h at 4 °C. Reactions were terminated by rapid filtration using a Brandel cell harvester onto Whatman GF/C filters and the trapped radioactivity was measured. Non-specific binding was defined as the binding in the presence of 30 μM phentolamine.

Functional study

A prostate tissue strip was set up in an organ bath containing modified Krebs–Henseleit solution aerated with 95% O_2 and 5% CO_2 at 37 °C and the isometric tension change was measured (Morishima *et al*., 2007b). Desipramine (0.1 μM), deoxycorticosterone (5 μM) and propranolol (1 μM) were added to the bathing solution to block neural and extraneural uptake of noradrenaline and to block β-adrenocep-

tors. Antagonists were added to the bath 45 min before and during the evaluation of cumulative concentration–response curves for noradrenaline.

Data analysis

As described previously (Muramatsu *et al*., 2005; Morishima *et al*., 2008), binding data were analysed with Graph Pad PRISM software (Ver. 3, Graph Pad Software, San Diego, CA, USA).

The abundance of α_1-adrenoceptor was expressed as the maximal binding capacity per mg of total tissue protein (B_{max}: fmol mg^{-1} of total tissue protein); that is, the binding amount in the crude membranes was divided by the total tissue protein (homogenate protein). In saturation binding studies, data were fitted by a one-site saturation binding isotherm. In competition studies, the data were first fitted to a one- and then a two-site model, and if the residual sums of squares were significantly less for a two-site fit of the data than for a one-site fit (P-value <0.05 as determined by F-test), then a two-site model was accepted.

In functional studies, antagonist affinity estimates (pK_B values) were obtained by plotting the data according to Arunlakshana and Schild (1959). When the straight lines yielded a slope with unity, the pA_2 value estimated was represented as the pK_B value. When a single concentration of antagonist was tested, the pK_B value was also determined for a single concentration of antagonist by the concentration ratio method (Furchgott, 1972).

Data are shown as the mean ± s.e.mean with the number of experiments. Values were compared by Student's t-test and P-values <0.05 were considered significant.

Drugs

The chemicals used were as follows: [^{3}H]-silodosin (1.92 TBq mmol^{-1}), silodosin (formerly known as KMD-3213), tamsulosin (from Kissei Pharmaceutical Co. Ltd., Matsumoto, Japan); [^{3}H]-prazosin (7-methoxy-[^{3}H]-prazosin, 2.74 TBq mmol^{-1}; Amersham, Buckinghamshire, UK), and bunazosin hydrochloride (Santen Co. Ltd, Osaka, Japan). Other drugs were purchased commercially.

Results

[^{3}H]-silodosin binding in intact segments and membrane preparations of rabbit prostate

[^{3}H]-silodosin (50–1000 pM) bound to intact segments of rabbit prostate in a concentration-dependent manner (Figure 1a). The specific binding was more than 60% of total binding at 1000 pM [^{3}H]-silodosin and the Hill coefficient was close to unity (0.93). Therefore, it was concluded that [^{3}H]-silodosin bound to a single class of sites. The

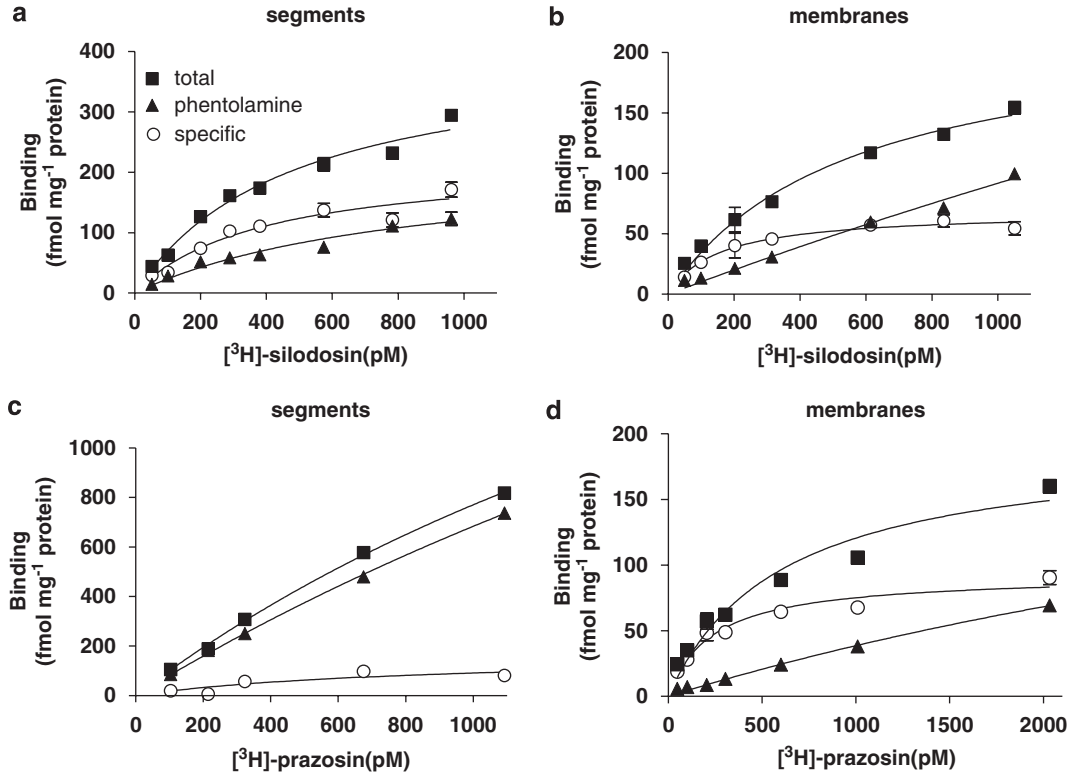

Figure 1 Binding of [^{3}H] silodosin (**a**, **b**) and [^{3}H]-prazosin (**c**, **d**) to rabbit prostate. Saturation binding curves in intact segments (**a**, **c**) and crude membranes (**b**, **d**) of rabbit prostate. The ordinate scale represents binding (fmol mg^{-1} total tissue protein). The specific binding was determined by subtracting the amount bound in the presence of 30 μM phentolamine (non-specific binding) from the total amount bound. Each point represents the mean of duplicate determinations. Each figure is representative of similar results obtained in four separate experiments.

dissociation constant (K_D) and maximal binding capacity (B_{max}) were 380 ± 30 pM and 210 ± 25 fmol mg^{-1} total tissue protein, respectively ($n = 5$). [^{3}H]-silodosin also bound to the crude membrane preparations of rabbit prostate, but the density was low ($B_{max} = 77 \pm 8$ fmol mg^{-1} total tissue protein, $K_D = 210 \pm 10$ pM, $n = 5$) (Figure 1b).

The pharmacological profiles of [^{3}H]-silodosin-binding sites were examined in competition binding studies using several antagonists. The competition curve for prazosin in the tissue segments was shallow, better fitting a two-site model by computer analysis (Figure 2a). The pKi values for prazosin at high- and low-affinity sites were 8.8 and 7.1, respectively, and the proportion of high affinity sites was 42% (Table 1). Two similar affinity sites were estimated in the competition curves for bunazosin, RS-17053 and 5-methylurapidil (Figure 2b), but not for silodosin, tamsulosin or BMY-7378. On the other hand, in the crude membrane preparations, the tested compounds showed monophasic competition curves (Figures 2a and b; Table 1), supporting a single component of [^{3}H]-silodosin binding sites in the membrane preparations.

[^{3}H]-prazosin binding in intact segments and membrane preparations of rabbit prostate

[^{3}H]-prazosin (50–1000 pM) also bound to intact segments of rabbit prostate. However, the proportion of specific binding was extremely low (less than 10% of total binding at 1000 pM [^{3}H]-prazosin, Figure 1c); thus, it was impossible to analyse the specific binding statistically. Nevertheless, in the crude membrane preparations, [^{3}H]-prazosin (50–2000 pM) generated significant binding with a high affinity (Figure 1d) ($K_D = 204 \pm 12$ pM, $B_{max} = 83 \pm 8$ fmol mg^{-1} total tissue protein, $n = 5$). The binding sites in crude membranes were competitively bound by prazosin, silodosin and 5-methylurapidil with high monophasic affinity (Figure 2c for silodosin and 5-methylurapidil, Table 1). Other drugs were not examined because of the limited amount of crude membranes.

Functional affinities for various α_1-adrenoceptor antagonists

Cumulative application of noradrenaline produced concentration-dependent contractions in the strips of rabbit prostate ($pEC_{50} = 6.0 \pm 0.1$, $n = 12$). This concentration–response curve was shifted to the right by relatively high concentrations of prazosin (0.1–1 μM, Figure 3a), 5-methylurapidil (0.1–1 μM, Figure 3b) or RS-17053 (1 μM). Thus, low pK_B values were estimated from the Schild plot analysis and concentration ratio method (Figure 3d, Table 2). In contrast, silodosin (Figure 3c) and tamsulosin at 3–10 nM produced an unsurmountable inhibition in the concentration–response curves for noradrenaline. BMY-7378 (1 μM) had no effect on the contraction.

Discussion

In the intact segments (strips) and membrane preparations of rabbit prostate, pharmacologically different profiles of α_1-adrenoceptors have been identified by radioligand binding and functional approaches. In this study, [^{3}H]-silodosin

Table 1 Binding affinities for various α_1-adrenoceptor antagonists estimated at [^{3}H]-silodosin and [^{3}H]-prazosin-binding sites in rabbit prostate

Drug	*[^{3}H]-silodosin*			*[^{3}H]-prazosin*
	Segments		*Membranes*	*Membranes*
	pK_i high (% high)	*pK_i low*	*pK_i*	*pK_i*
Prazosin	8.8 ± 0.1 (42 ± 4%)	7.1 ± 0.2	9.9 ± 0.2	9.8 ± 0.2
Bunazosin	9.1 ± 0.1 (34 ± 7%)	7.8 ± 0.2		
Silodosin	9.5 ± 0.1		9.8 ± 0.1	10.1 ± 0.1
Tamsulosin	9.7 ± 0.1			
RS-17053	9.6 ± 0.6 (27 ± 8%)	6.8 ± 0.3		
5-Methylurapidil	9.6 ± 0.2 (45 ± 5%)	7.6 ± 0.1	9.5 ± 0.3	9.8 ± 0.2
BMY-7378		5.2 ± 0.2		

Values from four or five experiments.
pK_i high and pK_i low: negative logarithm of equilibrium dissociation constants at high- and low-affinity sites for tested drugs.
% high: proportion of high-affinity sites.

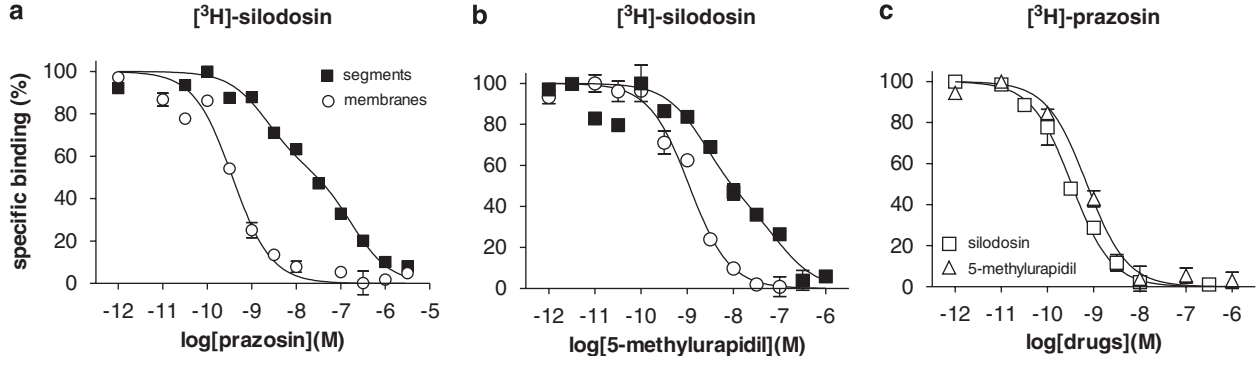

Figure 2 Competition curves for prazosin and 5-methylurapidil at [^{3}H]-silodosin-binding sites (**a**, **b**) and for silodosin and 5-methylurapidil at [^{3}H]-prazosin-binding sites (**c**) in rabbit prostate. In (**a**) and (**b**), 300 pM [^{3}H]-silodosin binding to intact segments and membranes was in competition with prazosin (**a**) and 5-methylurapidil (**b**). In (**c**), 500 pM [^{3}H]-prazosin binding to membranes was in competition with silodosin and 5-methylurapidil. Each figure is representative of similar results obtained in four separate experiments.

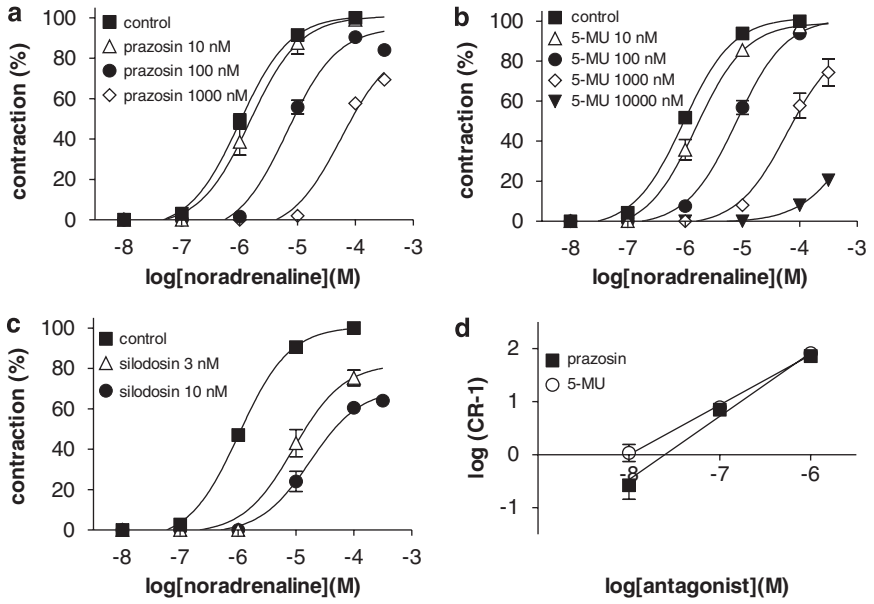

Figure 3 Concentration–response curves for noradrenaline in rabbit prostate. (**a**–**c**) Effects of prazosin, 5-methylurapidil (5-MU), and silodosin, respectively, on the concentration–response curves for noradrenaline. (**d**) Schild plot analysis for prazosin and 5-methylurapidil; their slopes were 1.15 ± 0.15 and 0.94 ± 0.14, respectively. Data show mean ± s.e.mean of 4–5 experiments.

Table 2 Functional affinities for various α_1-adrenoceptor antagonists estimated in contractile responses to noradrenaline in rabbit prostate

Drug	*pK_B*	*Slope (Schild analysis)*
Prazosin	7.6 ± 0.2	1.15
Bunazosin	7.9 ± 0.2	1.03
Silodosin	9.6 ± 0.1[a]	
Tamsulosin	9.6 ± 0.1[a]	
RS-17053	6.7 ± 0.3[a]	
5-Methylurapidil	8.0 ± 0.1	0.94
BMY-7378	NI	

Mean ± s.e. from four or five experiments.
NI: no inhibition at 1 μM BMY-7378.
[a]Estimated at 3 nM silodosin or tamsulosin and 1 μM RS-17053 by the concentration ratio method (Furchgott, 1972).

selectively bound to the α_1-adrenoceptors in the intact tissue segments, and the binding sites were composed of two different components with distinct affinities not only for prazosin, but also for other α_{1A}-adrenoceptor-selective antagonists (5-methylurapidil and RS-17053) (Ford *et al*., 1996; Morishima *et al*., 2008). However, the subtype-non-selective antagonist tamsulosin did not discriminate between components, and BMY-7378, an α_{1D}-adrenoceptor antagonist, showed a low affinity. According to the criteria of subclassification of α_1-adrenoceptors (Hieble *et al*., 1995; Ford *et al*., 1996; Morishima *et al*., 2007b; Alexander *et al*., 2008) and the selectivity of [^{3}H]-silodosin to α_{1A}- and α_{1L}-adrenoceptors (Morishima *et al*., 2008), it is likely that the [^{3}H]-silodosin-binding sites in the intact segments consist of α_{1L} subtype along with another subtype, designated here as 'α_{1A}-like' subtype because of its moderate affinity for prazosin (pKi = 8.8) and high affinities for other α_{1A} antagonists (silodosin, 5-methylurapidil and RS-17053). Such an α_{1L} profile, but not an α_{1A}-like profile, was also identified in the contractile response to noradrenaline, in which the intact strips of rabbit prostate were used. These results were consistent with those recently obtained in intact segments of human prostate (Morishima *et al*., 2007b).

In addition, this study further demonstrated that [^{3}H]-prazosin at subnanomolar concentrations did not bind significantly to the α_1-adrenoceptors in the intact segments of rabbit prostate (Figure 1c). This low capacity of specific binding seemed to be due to the low affinity for prazosin to the α_1-adrenoceptors in intact segments. This point was confirmed from the competition of prazosin at the [^{3}H]-silodosin-binding sites in the intact segments, from which two low-affinity constants were estimated for prazosin (pKi = 8.8 and 7.1) (Figure 2a, Table 1). An extremely high proportion of non-specific binding of [^{3}H]-prazosin (Figure 1c) made it impossible to estimate the specific binding and to use higher concentrations of [^{3}H]-prazosin. Thus, it seemed that two phenotypes consisting of α_{1L} subtypes and apparent α_{1A}-like subtypes coexisted in the intact segments of rabbit prostate, but that their affinities for prazosin were too low to define specific binding adequately with this ligand.

In contrast to the intact tissue segments, the crude membrane preparations of rabbit prostate bound not only [^{3}H]-silodosin but also [^{3}H]-prazosin with high affinities (K_D = 210 and 204 pM, respectively) and with the same densities (B_{max} = 77 and 83 fmol mg^{-1} total tissue protein for [^{3}H]-silodosin and [^{3}H]-prazosin, respectively). The binding sites of both radioligands were composed of a single class of α_1-adrenoceptor, and the pharmacological profile corresponded to the characteristic profile of classical α_{1A}-adrenoceptors (pK_i for prazosin = approximately 9.8) (Daniels *et al*., 1999; Ramsay *et al*., 2004). This suggested that two distinct α_1-adrenoceptor phenotypes occurring in intact segments became a single phenotype with a high affinity for prazosin

upon homogenization, resulting in significant specific binding at subnanomolar concentrations of [^{3}H]-prazosin. Thus, only one phenotype corresponding to classical α_{1A}-adrenoceptors was detected in the membrane preparations of rabbit prostate.

However, the densities of α_1-adrenoceptors detected in the membrane preparations were substantially lower than the B_{max} (210 fmol mg^{-1} total tissue protein) for [^{3}H]-silodosin binding in the intact segments. This represents a significant loss of α_1-adrenoceptors in the crude membranes. Such a loss of receptor after homogenization has also been reported in the α_1-adrenoceptors of blood vessels (Faber *et al*., 2001; Hiraizumi-Hiraoka *et al*., 2004; Tanaka *et al*., 2004) and prostate (Morishima *et al*., 2007b), the β-adrenoceptors of heart (Horinouchi *et al*. 2006) and the muscarinic acetylcholine receptors of urinary bladder (Anisuzzaman *et al*., 2008). However, no loss has been observed in the α_1-adrenoceptors and muscarinic acetylcholine receptors of cerebral cortex (Muramatsu *et al*., 2005; Morishima *et al*., 2008). Therefore, it is likely that the low yield of receptors after homogenization is not related to either the kind of receptors or the changes in phenotypes mentioned above. Rather, it may be dependent on the tissues, as such losses are more evident in fibrous tissues (Muramatsu *et al*., 2005).

Therefore, this study has identified three distinct pharmacological phenotypes of α_1-adrenoceptors in the rabbit prostate: α_{1L} phenotype with low affinity for prazosin ($pK_i = 7.1$) in the intact segments, α_{1A}-like phenotype with relatively low affinity for prazosin ($pK_i = 8.8$) in the intact segments and typical α_{1A} phenotype having the same profile as classical α_{1A}-adrenoceptor (pK_i for prazosin $= 9.8$) in the membranes. Ford's group have reported that recombinant α_{1A}-adrenoceptors behave functionally as the α_{1L} phenotype (Ford *et al*., 1997; Daniels *et al*., 1999). Our recent studies, including this one, have revealed that the α_{1L} phenotype converts into the classical α_{1A}-adrenoceptor after homogenization (Hiraizumi-Hiraoka *et al*., 2004; Morishima *et al*., 2007b, 2008). At the mRNA level, the α_{1A}-adrenoceptor was demonstrated to be the dominant α_1-adrenoceptor in the rabbit prostate (Piao *et al*., 2000), and α_{1B}- and α_{1D}-adrenoceptors were not detected in this and previous binding studies (Testa *et al*., 1993). Recently, we have found that the α_{1L}-adrenoceptor or its phenotype occurs in the wild-type mouse but is selectively abolished in α_{1A}-adrenoceptor gene knockout mice (Morishima *et al*., 2007a). These lines of evidence suggest that the three distinct phenotypes identified in the rabbit prostate and under independent assay conditions are not genetically different subtypes; rather they are more likely to have originated from a single α_{1A}-adrenoceptor gene product.

Recently, it has been suggested that antagonist affinity may not necessarily remain constant between tissues expressing the same receptors, especially under different assay conditions (Kenakin *et al*., 1995; Muramatsu *et al*., 2005; Nelson and Challiss, 2007). Among the three different phenotypes observed in this study, two distinct α_{1A} phenotypes may be simply accounted for by a change in receptor environment between intact segments and membranes (Muramatsu *et al*., 2005). However, another α_{1L} phenotype was identified as an entity, completely distinct from the α_{1A}-like phenotype in the same tissue segments and under the same assay conditions. Therefore, if both α_{1L} and α_{1A}-like phenotypes originate from a single gene, additional mechanisms or factors that would display the α_{1L} profile may be involved in the expression. We are now exploring these underlying mechanisms.

How many phenotypes exist or are detected in native tissues? The binding and functional affinities (pK_i/pK_B) of α_{1L} phenotype for prazosin were 7.1/7.6∼8.1 in the rabbit prostate (this study; Testa *et al*., 1997; van der Graaf *et al*., 1997), which were slightly lower than the affinities (8.3/8.4∼8.7) estimated in the human prostate (Ford *et al*., 1996; Morishima *et al*., 2007b) and those (8.3/7.9) in the rabbit ear artery (Hiraizumi-Hiraoka *et al*., 2004); however, the affinities for prazosin were higher than the affinities (6.5/6.7) estimated in the rabbit iris dilator (I Muramatsu *et al*., unpublished data). On the other hand, the affinities for some antagonists such as RS-17053 are relatively consistent with α_{1L} phenotypes of the rabbit and human prostates and the rabbit ear artery. These results suggest that there may be wide variation in the pharmacological profile among α_{1L} phenotypes (and probably other α_1-adrenoceptor phenotypes or subtypes) identified in many native tissues and species, and that the different pharmacological profiles may be distinguished by a subset of ligands. At this point, it is interesting to remember that a relatively wide range of functional affinities estimated for several antagonists was originally categorized into one subtype (group) in primary α_1-adrenoceptor subclassification (Drew, 1985; Flavahan and Vanhoutte, 1986; Muramatsu *et al*., 1990).

There has been significant controversy regarding the identity of α_1-adrenoceptors mediating prostatic contraction. This appears to be mainly due to a discrepancy between α_1-adrenoceptor profiles obtained by the bioassay approach (α_{1L} phenotype) and the membrane binding approach (classical α_{1A}-adrenoceptor). However, this study clearly shows that the classical α_{1A} phenotype does not occur in the intact prostatic segments and that there is a good concordance between the functional α_{1L} phenotype and binding α_{1L} phenotype, both of which were obtained from intact strips or segments. These results strongly suggest that the α_{1L} phenotype is a functional α_1-adrenoceptor involved in prostatic contraction and is a major target of α_1-adrenoceptor antagonist therapy of urinary outlet obstruction in patients with benign prostatic hyperplasia (Lepor and Shapiro, 1994; Cooper *et al*., 1999; Chapple, 2001; Andersson, 2002).

In conclusion, three pharmacologically distinct phenotypes of α_1-adrenoceptor were detected in the rabbit prostate, and all of the phenotypes appeared to be derived from a single α_{1A}-adrenoceptor gene. Among them, only the α_{1L} phenotype occurring in intact tissue segments is involved in prostatic contraction.

Note added in proof: Another paper in press has also dealt with this question: Gray KT, Short JL, Ventura S (2008). The α_{1A}-adrenoceptor gene is required for the α_{1L}-adrenoceptor-mediated response in isolated preparations of the mouse prostate. *Br J Pharmacol* **155**: 103–109.

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Conflict of interest

The authors state no conflict of interest.

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