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RESEARCH PAPER

Expression of distinct α_1-adrenoceptor phenotypes in the iris of pigmented and albino rabbits

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Background and purpose: The expression of multiple pharmacological phenotypes including α_{1L}-adrenoceptor has recently been reported for α_1-adrenoceptors. The purpose of the present study was to identify α_1-adrenoceptor phenotypes in the irises of pigmented and albino rabbits.
Experimental approach: Radioligand binding and functional bioassay experiments were performed in segments or strips of iris of pigmented and albino rabbits, and their pharmacological profiles were compared.
Key results: [^{3}H]-silodosin at subnanomolar concentrations bound to intact segments of iris of pigmented and albino rabbits at similar densities (approximately 240 fmol·mg^{-1} protein). The binding sites in the iris of a pigmented rabbit were composed of a single component showing extremely low affinities for prazosin, hydrochloride [*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethamine hydrochloride (RS-17053)] and 5-methylurapidil, while two components with high and low affinities for prazosin, RS-17053 and 5-methylurapidil were identified in irises from albino rabbits. In contrast, specific binding sites for [^{3}H]-prazosin were not clearly detected because a high proportion of non-specific binding and/or low affinity for prazosin occurred. Contractile responses of iris dilator muscle to noradrenaline were antagonized by the above ligands, and their antagonist affinities were consistent with the binding estimates at low-affinity sites identified in both strains of rabbits.
Conclusions and implications: A typical α_{1L} phenotype with extremely low affinity for prazosin is exclusively expressed in the iris of pigmented rabbits, while two distinct phenotypes (α_{1A} and α_{1L}) with high and moderate affinities for prazosin are co-expressed in the iris of albino rabbits. This suggests that a significant difference in the expression of phenotypes of the α_1-adrenoceptor occurs in the irises between the two strains of rabbits.
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Keywords: α_{1L}-adrenoceptor; α_{1A}-adrenoceptor; phenotype pharmacology; rabbit iris; pigmented and albino rabbits

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

Introduction

Three distinct subtypes of α_1-adrenoceptor (α_{1A}, α_{1B} and α_{1D}; nomenclature follows Alexander *et al.*, 2008) have been cloned, and their specific pharmacological profiles are recognized not only for the recombinant receptors but also for the native receptors of many tissues (Lomasney *et al.*, 1991; Hieble *et al.*, 1995; Michelotti *et al.*, 2000). The three classical α_1-adrenoceptor subtypes show high (subnanomolar) affinity for prazosin, a typical selective α_1-adrenoceptor antagonist. On the other hand, unique α_1-adrenoceptors showing low affinities for prazosin, originally found in functional studies of blood vessels and lower urinary tract, have indicated the

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presence of an additional α_1-adrenoceptor subtype (α_{1L}-adrenoceptor) (Flavahan and Vanhoutte, 1986; Muramatsu *et al*., 1990; Ford *et al*., 1996). However, in recent studies with α_1-adrenoceptor gene knockout mice, it was demonstrated that the α_{1L}-adrenoceptor is not genetically different from the classical α_1-adrenoceptor subtypes and rather is likely to be a different phenotype of the α_{1A}-adrenoceptor (Gray *et al*., 2008; Muramatsu *et al*., 2008). Thus, it is now thought that α_1-adrenoceptors should be classified into not only genome-based but also phenotype-based subtypes (Muramatsu *et al*., 2008; Su *et al*., 2008).

It is well known that the iris dilator muscle is sympathetically innervated through α_1-adrenoceptors. The α_1-adrenoceptor mediating the contraction of rabbit iris dilator muscle has a low affinity for prazosin (α_1 subtype) (Ishikawa *et al*., 1996; Nakamura *et al*., 1999), while, at the mRNA level, the α_1-adrenoceptor subtype predominantly expressed is α_{1A} (Suzuki *et al*., 2002), and, in radioligand binding experiments with the membrane preparations, the pharmacological profile identified α_{1A}-adrenoceptors (Nakamura *et al*., 1999; Suzuki *et al*., 2002). These contradictory reports on the α_1-adrenoceptor profiles in the rabbit iris dilator muscle may now be interpreted without confusion because it has been demonstrated that both the α_{1L}- and α_{1A}-adrenoceptor subtypes are derived from the same α_{1A}-adrenoceptor gene, as mentioned above, and that the pharmacological profile of the α_{1L}-adrenoceptor can convert to the α_{1A}-profile upon tissue homogenization (Hiraizumi-Hiraoka *et al*., 2004; Morishima *et al*., 2007; 2008; Muramatsu *et al*., 2008; Su *et al*., 2008). However, a functional study demonstrated a significant difference in the α_{1L}-adrenoceptor affinities for prazosin between irises of albino and pigmented rabbits (Ishikawa *et al*., 1996). In the present study, we reinvestigated α_{1L}-adrenoceptors in the irises from pigmented and albino rabbits and found that the pharmacological phenotype observed in the iris of pigmented rabbits is quite distinct from the α_1-adrenoceptors in the irises from albino rabbits.

Methods

Animals

Male Dutch pigmented rabbits (1.5–2 kg) and Japanese albino rabbits (2–3 kg) were anaesthetized with sodium pentobarbital (100 mg·kg^{-1}) and killed. The eyes were isolated and cleaned in a modified Krebs–Henseleit solution (composition in mmol·L^{-1}: NaCl, 120.7; KCl, 5.9; $MgCl_2$, 1.2; $CaCl_2$, 2.0; NaH_2PO_4, 1.2; $NaHCO_3$, 25.5; and D-glucose, 11.5; pH 7.4) aerated with 95% O_2 and 5% CO_2. The present study was performed according to the Guidelines for Animal Experiments, University of Fukui.

Tissue segment binding experiments with rabbit irises

Tissue segment binding experiments were performed as described previously (Muramatsu *et al*., 2005; Morishima *et al*., 2008). By using a dissecting microscope, the iris of one eye was cut into 10 segments without separating the ciliary body and ciliary process. That is, the iris segments used in the binding experiments included the iris, ciliary process and ciliary body. Twenty segments were prepared from one rabbit and used for one saturation or competition experiment. Each iris segment was incubated with [^{3}H]-silodosin or [^{3}H]-prazosin for 16 h at 4°C in 1 mL of a Krebs incubation buffer containing 135.7 mmol·L^{-1} NaCl, 5.9 mmol·L^{-1} KCl, 1.2 mmol·L^{-1} $MgCl_2$, 2.0 mmol·L^{-1} $CaCl_2$, 1.2 mmol·L^{-1} NaH_2PO_4, 10.5 mmol·L^{-1} $NaHCO_3$ and 11.5 mmol·L^{-1} D-glucose (pH 7.4). In binding saturation experiments, [^{3}H]-silodosin or [^{3}H]-prazosin at concentrations between 50 and 1000 pmol·L^{-1} was used. Binding competition experiments were performed with 500 pmol·L^{-1} [^{3}H]-silodosin. After incubation, the pieces were gently washed at 4°C and then dissolved in 1 mL of 0.3 mol·L^{-1} NaOH solution before the radioactivity and protein content were estimated. The specific binding was determined by subtracting the non-specific binding measured in the presence of 30 μmol·L^{-1} phentolamine from the total radioactivity-bound·mg^{-1} protein.

Functional studies with iris dilator muscle

Functional studies were performed as described previously (Nakamura *et al*., 1999). Briefly, rabbit iris strips were placed at 37°C in organ baths containing a modified Krebs–Henseleit solution composed of (in mmol·L^{-1}): NaCl, 120.7; KCl, 5.9; $MgCl_2$, 1.2; $CaCl_2$, 2.0; NaH_2PO_4, 1.2; $NaHCO_3$, 25.5; and D-glucose, 11.5. Noradrenaline was applied cumulatively, and the isometric tension changes of dilator muscle were recorded through a force transducer. Desipramine (0.3 μmol·L^{-1}), deoxycorticosterone acetate (5 μmol·L^{-1}) and propranolol (1 μmol·L^{-1}) were added to inhibit neural and extraneural reuptake of noradrenaline and to block β-adrenoceptors, as described previously (Muramatsu *et al*., 1995). Antagonists were applied 40 min before and during the evaluation of contractile responses to noradrenaline.

Data analysis

Data are presented as the mean ± standard error of the mean (SEM) of a number of experiments. Data were statistically analysed by Student's *t*-test.

Binding data in saturation and competition experiments were analysed by using PRISM software (ver. 3, GraphPad, San Diego, CA, USA). The number of α_1-adrenoceptors was presented as the maximum binding capacity·mg^{-1} of total tissue protein (B_{max}: fmol·mg^{-1} of total tissue 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 square sums were significantly lower for a two-site fit of the data than for a one-site fit ($P < 0.05$, as determined by *F*-test), then a two-site model was accepted. Slopes of pseudo-Hill plots were also determined for some competitors to validate one- or two-site fitting. For pseudo-Hill plot analyses, Origin software (ver 7.5, Origin Lab Co., Northampton, MA, USA) was used.

In functional studies, antagonist affinity estimates (pK_B values) were obtained by plotting the data, according to Schild analysis. When the straight lines had a slope of unity, the pA_2 value estimated was taken as the pK_B value. When a single concentration of an antagonist was tested, the pK_B

value was also determined for a single concentration of the antagonist by the concentration-ratio method (Furchgott, 1972).

Drugs

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

Results

[^{3}H]-silodosin and [^{3}H]-prazosin binding in iris segments of pigment and albino rabbits

[^{3}H]-silodosin (50–1000 pmol·L^{-1}) bound to the iris segments of pigmented and albino rabbits in a concentration-dependent manner (Figure 1A,C). The proportion of specific binding was relatively low, approximately 30% of total binding at 1000 pmol·L^{-1} [^{3}H]-silodosin and markedly reduced at higher concentrations of [^{3}H]-silodosin (data not shown). However, the variance among segments was small at the concentrations less than 1000 pmol·L^{-1}, and the Hill coefficients were estimated to be close to unity (0.93 ± 0.06 and 0.97 ± 0.05 for pigmented and albino rabbits respectively). Therefore, we performed saturation binding experiments at concentrations ranging from 50 to 1000 pmol·L^{-1} and concluded that [^{3}H]-silodosin bound to a single class of sites in the iris segments of both strains of rabbits. The binding parameters are summarized in Table 1. The dissociation constant (pK_D) and B_{max} in the iris segments were not significantly different between pigmented and albino rabbits.

In contrast to [^{3}H]-silodosin binding, the non-specific binding of [^{3}H]-prazosin was extremely high (Figure 1B,D). In particular, specific binding of [^{3}H]-prazosin was not detected in the iris segments from the pigmented rabbits. In three out

Table 1 Binding parameters of [^{3}H]-silodosin in iris segments of pigmented and albino rabbits

	[^{3}H]-silodosin	
	B_{Max} (fmol·mg^{-1} protein)	pK_D
Pigmented rabbit	246 ± 26	9.2 ± 0.1
Albino rabbit	229 ± 16	9.1 ± 0.1

Data shown are means ± SEM from five experiments.
SEM, standard error of the mean; B_{max}, maximum binding capacity.

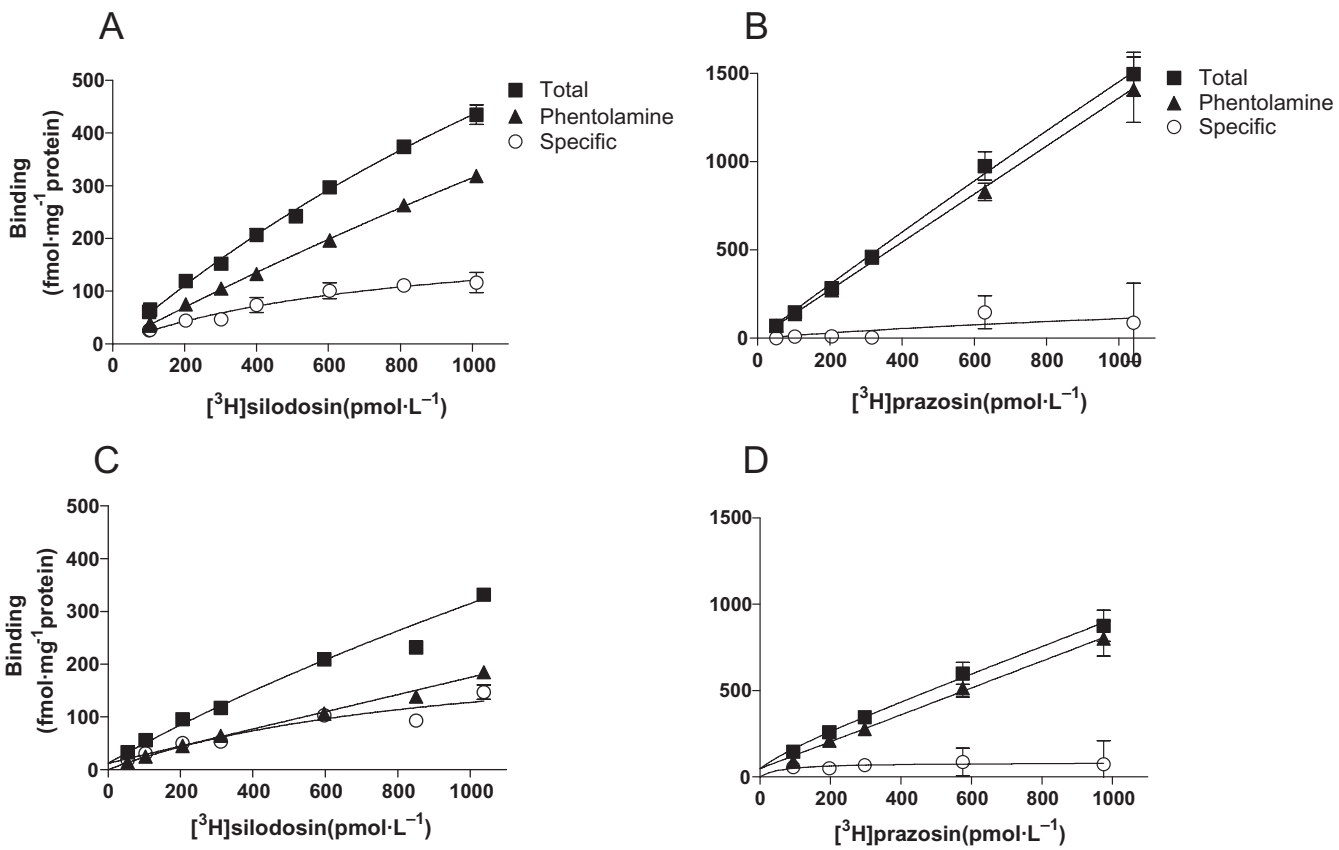

Figure 1 Binding of [^{3}H]-silodosin (A, C) and [^{3}H]-prazosin (B, D) to iris segments of pigmented (A, B) and albino (C, D) rabbits. The ordinate scale represents binding (fmol·mg^{-1} total tissue protein). The specific binding was determined by subtracting the amount bound in the presence of 30 μmol·L^{-1} phentolamine (non-specific binding) from the total amount bound. Each point represents the mean ± SEM of five experiments. SEM, standard error of the mean.

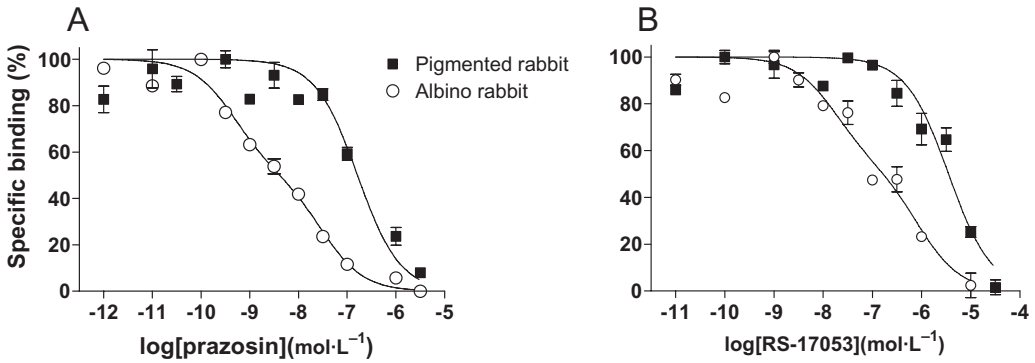

Figure 2 Competition curves for prazosin and RS-17053 at [^{3}H]-silodosin binding sites in intact segments of rabbit iris. Binding of 500 pmol·L^{-1} [^{3}H]-silodosin was in competition with prazosin (A) and RS-17053 (B). Each point is representative of similar results obtained in four separate experiments. RS-17053, *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethamine hydrochloride.

Table 2 Binding affinities for various α_1-adrenoceptor antagonists estimated at [^{3}H]-silodosin binding sites in iris segments of pigmented and albino rabbits

	Pigmented rabbit	*Albino rabbit*
	pKi	*pKi$_{high}$ (% high) pKi$_{low}$*
Prazosin	6.3 ± 0.3	9.5 ± 0.3 (50) 7.6 ± 0.2
Bunazosin	6.2 ± 0.1	9.3 ± 0.2 (52) 6.4 ± 0.3
RS-17053	5.7 ± 0.3	8.3 ± 0.2 (43) 6.5 ± 0.1
Silodosin	9.0 ± 0.2	ND
Tamsulosin	9.1 ± 0.3	9.6 ± 0.4
5-Methylurapidil	7.5 ± 0.1	9.1 ± 0.2 (47) 7.4 ± 0.2
BMY 7378	6.2 ± 0.1	ND

Data shown are means ± SEM of four or five experiments. pKi$_{high}$ and pKi$_{low}$: negative logarithm of equilibrium dissociation constants at high- and low-affinity sites for antagonists tested. % high: proportion of high-affinity sites. BMY 7378, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5] decane-7,9-dione dihydrochloride; ND, not determined; SEM, standard error of the mean.

of five albino rabbits, specific [^{3}H]-prazosin binding sites could be determined. B_{max} value was found to be 94 ± 20 fmol·mg^{-1} protein, but no specific binding was clearly detected in the remaining two albino rabbits. Therefore, only the [^{3}H]-silodosin binding sites were characterized in the following experiments.

Figure 2 shows representative competition curves for prazosin and *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethamine hydrochloride (RS-17053) against [^{3}H]-silodosin binding in iris segments of pigmented and albino rabbits. Prazosin and RS-17053 competed for binding monotonically with low affinities in iris segments from the pigmented rabbit (Figure 2 and Table 2). In contrast to the binding in the pigmented rabbit, both ligands biphasically inhibited the binding in the segments from the albino rabbit. Thus, the [^{3}H]-silodosin binding sites of albino rabbit iris are composed of two components with different affinities for prazosin, RS-17053, bunazosin or 5-methylurapidil. The results of the competition experiments are summarized in Table 2. The binding affinities (pKi values) for each ligand tested were lower in iris segments from the pigmented rabbit than in those from albino rabbits (Table 2).

Contractile responses to noradrenaline in rabbit iris dilator muscle. Noradrenaline (0.01–100 μmol·L^{-1}) produced a concentration-dependent contraction in iris dilator muscle (EC_{50}: 0.30 ± 0.10 and 0.52 ± 0.06 μmol·L^{-1} in pigmented and albino rabbits, respectively, n = 8). The contractile responses were antagonized by prazosin at concentrations greater than 0.1 μmol·L^{-1}, resulting in shifts of the concentration-response curves to the right (Figure 3A,B). The effects of 5-methylurapidil in muscles from pigmented and albino rabbits are also shown in Figure 3C,D. The functional affinities of several antagonists and the Schild slopes are summarized in Table 3. The potencies of prazosin, bunazosin, RS-17053, tamsulosi and 5-methylurapidil in antagonizing the response to noradrenaline were slightly higher in the dilator muscles from the albino rabbits than in those from the pigmented rabbits.

Discussion

The main functional α_1-adrenoceptor subtype in the iris dilator muscle has been characterized as the α_{1L} subtype, based on observations from functional studies, where the noradrenaline-induced contraction of iris dilator muscle was weakly antagonized by prazosin (Ishikawa *et al.*, 1996; Nakamura *et al.*, 1999). The assumption that the α_{1L} subtype is the chief subtype in iris is also supported by findings that α_{1A} is the main subtype expressed at the mRNA level and is the main subtype detected in the membrane binding studies because the α_{1L} and α_{1A} subtypes are now understood to originate from the same α_{1A}-adrenoceptor gene.

Typically, the pA_2 or pK_B values of prazosin for α_{1L}-adrenoceptors in many tissues such as human and rabbit prostate are around 8.0. However, in this study, we found that the pK_B value of prazosin for noradrenaline-induced contraction in the iris of the pigmented rabbit was especially low, 6.7 ± 0.1. Interestingly, the pK_B value of prazosin in albino rabbit was 7.8 ± 0.2, which is comparable with that for 'typical' α_{1L} subtypes.

To further identify the pharmacological profile of this atypical subtype, the tissue segment binding studies using irises from pigmented rabbits were conducted because this method

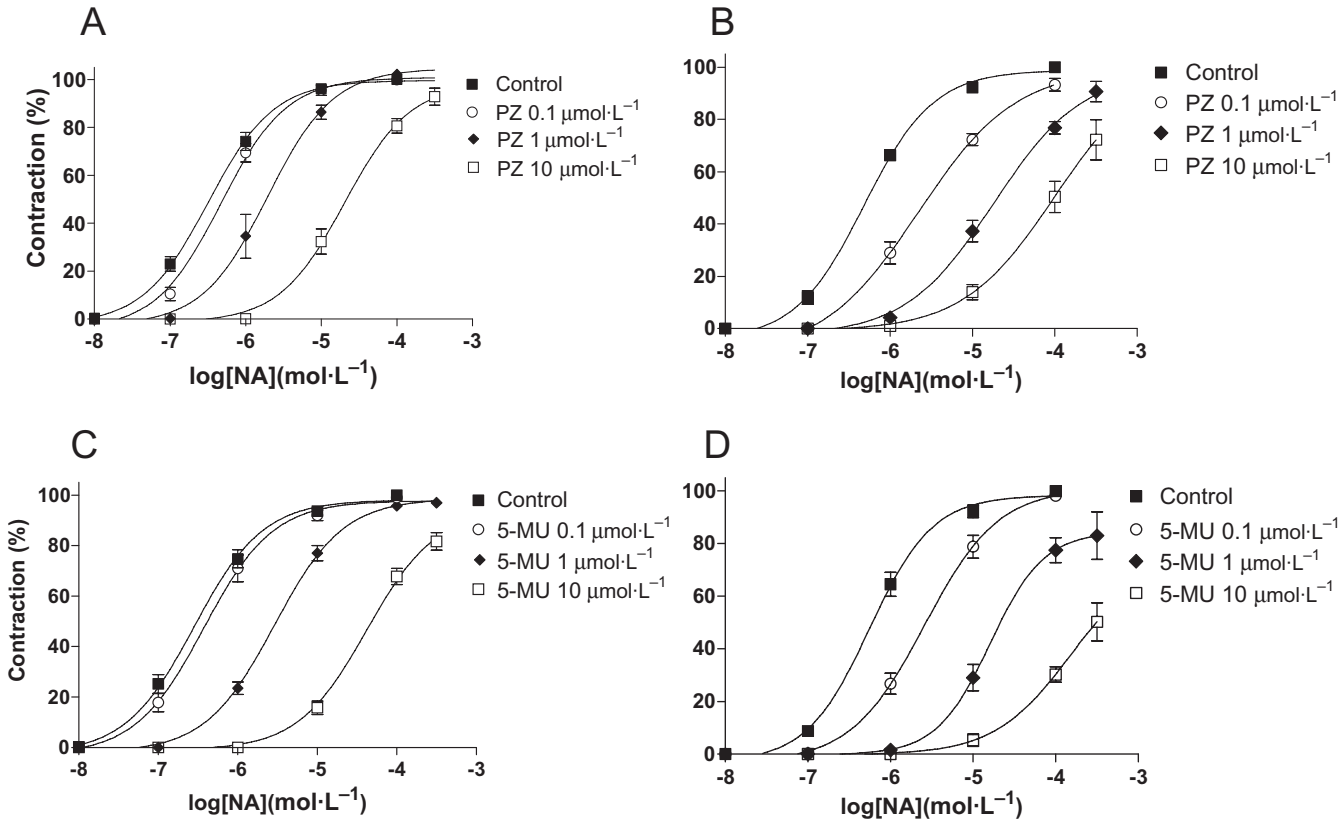

Figure 3 Effects of prazosin (PZ) and 5-methylurapidil (5-MU) on the concentration-response curves for noradrenaline (NA) in rabbit iris dilator muscle. (A, C) Pigmented rabbit. (B, D) Albino rabbit. Each point represents the mean ± SEM of five or six experiments. SEM, standard error of the mean.

Table 3 Functional affinities for various α_1-adrenoceptor antagonists estimated in contractile responses to noradrenaline in rabbit iris dilator

	Pigmented rabbit	*Albino rabbit*
	pK_B (slope)	*pK_B (slope)*
Prazosin	6.7 ± 0.1 (0.99)	7.8 ± 0.2 (0.86)
Bunazosin	7.0 ± 0.1 (1.11)	7.7 ± 0.2 (1.16)
RS-17053	<6	6.3 ± 0.2[a]
Silodosin	9.0 ± 0.2 (1.04)	9.5 ± 0.2 (1.17)
Tamsulosin	9.2 ± 0.1 (1.09)	9.7 ± 0.1 (0.97)
5-Methylurapidil	6.8 ± 0.2 (1.17)	7.6 ± 0.2 (1.01)
BMY 7378	<6	<6

Data shown are means ± SEM from four or six experiments. Slope was calculated from Schild analysis, which was not significantly different from unity.
[a]Estimated at 1 μmol·L^{-1} RS-17053 by the concentration-ratio method (Furchgott, 1972).
BMY 7378, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride; SEM, standard error of the mean; RS-17053, *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethamine hydrochloride.

is thought to be useful for identifying the pharmacological profile of the α_{1L} subtype under the native tissue environment (Muramatsu *et al.*, 2005). Two different radioligands were applied: [^{3}H]-silodosin, which has a very high selectivity for both α_{1A}- and α_{1L}-adrenoceptors, and [^{3}H]-prazosin, which has a high affinity for the classical α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (Murata *et al.*, 1999; Morishima *et al.*, 2008). [^{3}H]-silodosin bound to the α_1-adrenoceptors, although the non-specific binding was relatively high. In contrast, [^{3}H]-prazosin was unable to recognize the α_1-adrenoceptors as its specific binding sites because the non-specific binding was extremely high (more than 90% of total binding) and the affinity of prazosin for α_1-adrenoceptors was especially low in the pigmented rabbit. The α_1-adrenoceptor densities estimated from [^{3}H]-silodosin binding were the same (approximately 240 fmol·mg^{-1} protein) in irises from pigmented and albino rabbits, but the pharmacological profiles were apparently different between the strains. In the iris of the pigmented rabbit, the binding sites were composed of a single component, which showed extremely low affinities for prazosin (pKi = 6.3), bunazosin (6.2), RS-17053 (5.7) and 5-methylurapidil (7.5). In contrast, the binding sites in the iris of albino rabbit were composed of two components that were discriminated by the ligands mentioned above (i.e. pKi values for prazosin = 9.5 and 7.6). Considering the subtype selectivity of silodosin, prazosin, RS-17053 and 5-methylurapidil (Ford *et al.*, 1996; Muramatsu *et al.*, 1998; Murata *et al.*, 1999; Morishima *et al.*, 2008), it may be roughly summarized that the α_1-adrenoceptors in the iris of the pigmented rabbit correspond to the α_{1L} subtype but that the α_1-adrenoceptors in albino rabbit are of α_{1A} and α_{1L} subtypes.

However, it is difficult to categorize the iris α_{1L}-adrenoceptors simply as a single class of receptor because the pharmacological profiles of both α_{1L}-adrenoceptors were significantly different. In particular, binding and functional

Table 4 Pharmacological profiles of α_{1A}-adrenoceptor phenotypes

	Phenotypes (pK_i or pK_B for prazosin)				
Tissue	*Binding in segments*[a]		*Binding in membranes*[a]	*Function in strips*[b]	*References*[c]
Rat tail artery	α_{1A} (9.8)		α_{1A} (9.3)	α_{1A} (9.3)	Lachnit *et al.*, 1997 (F); Tanaka *et al.*, 2004 (B); Morishima *et al.*, 2008 (B)
Cerebral cortex	α_{1A} (9.9)	α_{1L} (7.8)	α_{1A} (10.2)		Morishima *et al.*, 2008 (B)
Mouse cerebral cortex	α_{1A} (10.1)	α_{1L} (8.5)	α_{1A} (9.9)		Muramatsu *et al.*, 2008 (B)
Vas deferens	α_{1A} (9.9)	α_{1L} (8.1)		α_{1L} (7.7)	Muramatsu *et al.*, 2008 (B, F)
Human prostate	α_{1A} (10.6)	α_{1L} (8.3)	α_{1A} (9.8)	α_{1L} (8.4)	Ford *et al.*, 1996 (F); Morishima *et al.*, 2007 (B, F)
Albino rabbit ear artery	α_{1A} (9.9)	α_{1L} (8.3)	α_{1A} (9.8)	α_{1L} (7.9)	Hiraizumi-Hiraoka *et al.*, 2004 (B, F)
Prostate	α_{1A} (9.1)	α_{1L} (7.4)		α_{1L} (8.0)	I. Muramatsu, unpubl. obs. (B, F); Van der Graaf *et al.*, 1997 (F)
Iris	α_{1A} (9.5)	α_{1L} (7.6)	α_{1A} (9.3)	α_{1L} (8.3)	Present study (B, F); Ishikawa *et al.*, 1996 (F); Nakamura *et al.*, 1999 (F)
Pigmented rabbit prostate	$\alpha_{1A\text{-like}}$ (8.8)	α_{1L} (7.1)	α_{1A} (9.9)	α_{1L} (7.6)	Su *et al.*, 2008 (B, F)
Iris		α_{1L} (6.3)		α_{1L} (6.6)	Present study (B, F); Ishikawa *et al.*, 1996 (F)

[a]Affinity estimates for prazosin at [^{3}H]-silodosin binding sites in intact segments and membrane preparations of various tissues were listed.
[b]Functional data represent a mean value when the pK_B values were different among the quoted references.
[c]References from which binding (B) and functional (F) data were quoted.

affinities for prazosin and RS-17053 were approximately 10 times lower in the iris of the pigmented rabbit than in that from the albino rabbit. Such a difference in the functional affinity for prazosin between the irises of pigmented and albino rabbits (pK_B = 6.4 and 8.6 respectively) was also observed by Ishikawa *et al*. (1996). As mentioned above, the affinity values (pKi or pK_B) of prazosin for the α_{1L}-adrenoceptors obtained in many tissues, including the iris of the albino rabbit and prostate from the pigmented rabbit (Table 4), are around 8. Recently, Palea *et al*. (2008) also reported that, in pigmented rabbits, tamsulosin and alfuzosin were 30 times less potent as antagonists of phenylephrine-induced contractions in iris dilator muscle than in prostatic muscle. Thus, it is likely that the exceptionally low profile of some α_1 antagonists is specific for the α_{1L}-adrenoceptor of the iris of the pigmented rabbit.

Recently, it has been demonstrated that multiple α_{1A}-adrenoceptor phenotypes, including the α_{1L}-adrenoceptor, are derived from a single α_{1A}-adrenoceptor gene (Gray *et al*., 2008; Muramatsu *et al*., 2008; Su *et al*., 2008). Table 4 shows a variety of α_{1A}-adrenoceptor phenotypes reported so far in various tissues and species, identified by the intact-tissue segment binding approach with [^{3}H]-silodosin or by functional bioassay studies with intact tissue strips. The data obtained by the conventional binding approach with tissue homogenates or membrane preparations are listed; only a single α_{1A} profile was detected with [^{3}H]-silodosin after tissue homogenization. In most of the tissues listed, α_{1A} and α_{1L} phenotypes coexist under conditions where the tissue is kept intact, whereas a single phenotype was expressed in a rat tail artery (α_{1A} phenotype) and in the iris of the pigmented rabbit (α_{1L} phenotype). From these lines of evidence, it is likely that, even though distinct phenotypes originate from a single α_{1A}-adrenoceptor gene, the expression of each phenotype is strongly dependent on any modification of the tissue being studied, rather than only a simple variation of the α_{1A}-adrenoceptor protein. Furthermore, it is interesting to note that a single phenotype is mainly involved in the function of a tissue, even though two distinct phenotypes may coexist in the same tissue. Therefore, in the future, not only genome-based but also phenotype-based receptor subtypes must be considered as independent targets of drug therapy (Muramatsu *et al*., 2005; Nelson and Challiss, 2007; Su *et al*., 2008). However, the mechanisms underlying the expression of a specific phenotype and its functional dominance are still unknown and need to be explored in further studies.

Bunazosin was first developed as an antihypertensive drug based on its similarity to prazosin but, in Japan, it is also used to decrease intraocular pressure in patients with glaucoma. It relaxes the ciliary muscle and increases the aqueous outflow (Nishimura *et al*., 1993). Interestingly, the ocular pharmacology of bunazosin has been mostly examined in albino rabbits. As mentioned above and in Table 4, two distinct phenotypes (α_{1A} and α_{1L}) coexist in the iris segments of the albino rabbit. Iris segments in our present study included the ciliary body (muscle) and ciliary process in addition to iris tissue (see Methods). Because bunazosin reduces intraocular pressure without affecting pupil diameter in albino rabbits, Nishimura *et al*. suggested that intraocular pressure and mydriasis are regulated through distinct α_1-adrenoceptor subtypes. Thus, from these results, it is likely that, in iris dilator muscle in albino rabbits, bunazosin acts selectively on the α_1-adrenoceptor (probably α_{1A} phenotype) in ciliary muscle without affecting the α_{1L} phenotype. With regard to this point, it is also interesting to note that, in pigmented rabbits, the effects of bunazosin on intraocular pressure and pupil diameter are negligible (Aihara *et al*., 1994; Ichikawa *et al*., 2004).

In summary, the results from the present study clearly show that an atypical α_{1L} phenotype having extremely low affinity for prazosin is expressed in the iris of the pigmented rabbit, and two distinct phenotypes (α_{1A} and α_{1L}) with high and moderate affinities for prazosin are expressed in the iris of the albino rabbit. The results of the present and previous studies strongly suggest that there is a large variation in the expression of α_{1A}-adrenoceptor phenotypes.

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Conflict of interest

The authors have no conflict of interest.

References

Aihara M, Araie M, Kaburaki T, Shirato S (1994). Effects of long-term application of bunazosin hydrochloride eye drops on the aqueous flow rate and blood-aqueous barrier permeability in rabbit eyes. *J Jpn Ophthalmol Soc* **98**: 540–544 (abstract in English).

Alexander SPH, Mathie A, Peters JA (2008). Guide to receptors and channels (GRAC), 3rd edn. *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Flavahan NA, Vanhoutte PM (1986). Alpha-1 and alpha-2 adrenoceptor: response coupling in canine saphenous and femoral veins. *Trends Pharmacol Sci* **7**: 347–349.

Ford AP, Arredondo NF, Blue DR Jr, Bonhaus DW, Jasper J, Kava MS *et al*. (1996). RS-17053 (N-[2-(2-cyclopropylmethoxy phenoxy)ethyl]-5-chloro-alpha, alpha-dimethyl-1H-indole-3-ethanamine hydrochloride), a selective alpha 1A-adrenoceptor antagonist, displays low affinity for functional alpha 1-adrenoceptors in human prostate: implications for adrenoceptor classification. *Mol Pharmacol* **49**: 209–215.

Furchgott RF (1972). The classification on adrenoceptors (adrenergic receptors): an evaluation from the standpoint of receptor theory. In: Blaschko H, Muscholl E (eds). *Handbuch der Experimentellen Pharmacology*, Vol. 3. Springer: New York, pp. 283–335.

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.

Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz RJ *et al*. (1995). International Union of Pharmacology. X. Recommendation for nomenclature of alpha 1-adrenoceptors: consensus update. *Pharmacol Rev* **47**: 267–270.

Hiraizumi-Hiraoka Y, Tanaka T, Yamamoto H, Suzuki F, Muramatsu I (2004). Identification of alpha-1L adrenoceptor in rabbit ear artery. *J Pharmacol Exp Ther* **310**: 995–1002.

Ishikawa H, Miller DD, Patil PN (1996). Comparison of postjunctional α_1-adrenoceptors in iris dilator muscle of humans, and albino and pigmented rabbits. *Naunyn Schmiedebergs Arch Pharmacol* **354**: 765–772.

Ichikawa M, Okada Y, Asai Y, Hara H, Ishii K, Araie M (2004). Effects of topically instilled bunazosin, an α_1-adrenoceptor antagonist, on constrictions induced by phenylephrine and ET-1 in rabbit retinal arteries. *Invest Ophtalmol Vis Sci* **45**: 4041–4048.

Lachnit WG, Tran AM, Clarke DE, Ford APDW (1997). Pharmacological characterization of an alpha 1A-adrenoceptor mediating contractile responses to noradrenaline in isolated caudal artery of rat. *Br J Pharmacol* **120**: 819–826.

Lomasney JW, Cotecchia S, Lefkowitz RJ, Caron MG (1991). Molecular biology of alpha-adrenergic receptors: implications for receptor classification and for structure-function relationships. *Biochim Biophys Acta* **1095**: 127–139.

Michelotti GA, Price DT, Schwinn DA (2000). Alpha-1 adrenergic receptor regulation: basic science and clinical implications. *Pharmacol Ther* **88**: 281–309.

Morishima S, Suzuki F, Yoshiki H, Anisuzzaman AS, Sathi ZS, Tanaka T *et al*. (2008). Identification of the alpha(1L)-adrenoceptor in rat cerebral cortex and possible relationship between alpha(1L)- and alpha(1A)-adrenoceptors. *Br J Pharmacol* **153**: 1485–1494.

Muramatsu I, Morishima S, Suzuki F, Yoshiki H, Anisuzzaman ASM, Tanaka T *et al*. (2008). Identification of α_{1L}-adrenoceptor in mice and its abolition by α_{1A}-adrenoceptor gene knockout. *Br J Pharmacol* **155**: 1224–1234.

Morishima S, Tanaka T, Yamamoto H, Suzuki F, Akino H, Yokoyama O *et al*. (2007). Identification of alpha-1L and alpha-1A adrenoceptors in human prostate by tissue segment binding. *J Urol* **177**: 377–381.

Muramatsu I, Ohmura T, Kigoshi S, Hashimoto S, Oshita M (1990). Pharmacological subclassification of alpha 1-adrenoceptors in vascular smooth muscle. *Br J Pharmacol* **99**: 197–201.

Muramatsu I, Ohmura T, Kigoshi S (1995). Pharmacological profiles of a novel alpha 1-adrenoceptor agonist, PNO-49B, at alpha 1-adrenoceptor subtypes. *Naunyn Schmiedebergs Arch Pharmacol* **351**: 2–9.

Muramatsu I, Murata S, Isaka M, Piao HL, Zhu J, Suzuki F *et al*. (1998). Alpha1-adrenoceptor subtypes and two receptor systems in vascular tissues. *Life Sci* **62**: 1461–1465.

Muramatsu I, Tanaka T, Suzuki F, Li Z, Hiraizumi-Hiraoka Y, Anisuzzaman AS *et al*. (2005). Quantifying receptor properties: the tissue segment binding method – a powerful tool for the pharmacome analysis of native receptors. *J Pharmacol Sci* **98**: 331–339.

Murata S, Taniguchi T, Muramatsu I (1999). Pharmacological analysis of the novel, selective alpha1-adrenoceptor antagonist, KMD-3213, and its suitability as a tritiated radioligand. *Br J Pharmacol* **127**: 19–26.

Nakamura S, Taniguchi T, Suzuki F, Akagi Y, Muramatsu I (1999). Evaluation of α1-adrenoceptors in the rabbit iris: pharmacological characterization and expression of mRNA. *Br J Pharmacol* **127**: 1367–1374.

Nelson CP, Challiss RA (2007). Phenotypic' pharmacology: the influence of cellular environment on G protein-coupled receptor antagonist and inverse agonist pharmacology. *Biochem Pharmacol* **73**: 737–751.

Nishimura K, Kuwayama Y, Matsugi T, Sun N, Shirasawa E (1993). Selective suppression by bunazosin of alpha-adrenergic agonist evoked elevation of intraocular pressure in sympathectomized rabbit eyes. *Invest Ophtalmol Vis Sci* **34**: 1761–1766.

Palea S, Chang DF, Rekik M, Regnier A, Liuel P (2008). Comparative effect of alfuzosin and tamsulosin on the contractile response of isolated rabbit prostatic and iris dilator smooth muscles. *J Cataract Refract Surg* **34**: 489–496.

Su TH, Morishima S, Suzuki F, Yoshiki H, Anisuzzaman ASM, Tanaka T *et al*. (2008). Native profiles of α_{1A}-adrenoceptor phenotypes in rabbit prostate. *Br J Pharmacol* **155**: 906–912.

Suzuki F, Taniguchi T, Nakamura S, Akagi Y, Kubota C, Satoh M *et al*. (2002). Distribution of alpha-1 adrenoceptor subtypes in RNA and protein in rabbit eyes. *Br J Pharmacol* **135**: 600–608.

Tanaka T, Zhang L, Suzuki F, Muramatsu I (2004). Alpha-1 adrenoceptors: evaluation of receptor subtype-binding kinetics in intact arterial tissues and comparison with membrane binding. *Br J Pharmacol* **141**: 468–476.

Van der Graaf PH, Deplanne V, Duquenne C, Angel I (1997). Analysis of alpha1-adrenoceptors in rabbit lower urinary tract and mesenteric artery. *Eur J Pharmacol* **327**: 25–32.