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Pharmacological characterization of two distinct α_1-adrenoceptor subtypes in rabbit thoracic aorta

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1 α_1-Adrenoceptor subtypes in rabbit thoracic aorta have been examined in binding and functional experiments.

2 [^{3}H]-prazosin bound to two distinct populations of α_1-adrenoceptors ($pK_{D,high} = 9.94$, $R_{high} = 79.2$ fmol mg^{-1} protein; $pK_{D,low} = 8.59$, $R_{low} = 215$ fmol mg^{-1} protein). Pretreatment with chloroethylclonidine (CEC, 10 μM) almost inactivated the prazosin-high affinity sites and reduced the number of the low affinity sites without changing the pK_D value.

3 In the displacement experiments with CEC-untreated membranes, unlabelled prazosin, WB4101 and HV723 displaced the binding of 200 pM [^{3}H]-prazosin monophasically; the affinities for WB4101 ($pK_I = 8.88$) and HV723 (8.49) were about 10 times lower than that for prazosin (9.99). In the CEC-pretreated membranes also, the antagonists inhibited the binding of 1000 pM [^{3}H]-prazosin monophasically; the pK_I values for prazosin, WB4101 and HV723 were 9.09, 8.97 and 8.17, respectively. These results suggest that the prazosin-high and low affinity sites can be independently appraised in the former and latter experimental conditions. Noradrenaline, but not methoxamine, showed slightly higher affinity for the prazosin-high affinity site than for the low affinity site.

4 In the functional experiments, noradrenaline (0.001–100 μM) and methoxamine (0.1–100 μM) produced concentration-dependent contractions. Pretreatment with CEC inhibited the contractions induced by low concentrations of noradrenaline but without effect on the responses to methoxamine. Prazosin inhibited the concentration-response curves for noradrenaline in the CEC-untreated aorta in a manner which was not consistent with competitive antagonism at a single site, and two distinct affinity constants ($pK_B = 9.71$ and 8.74) were obtained. However, after CEC-pretreatment, Schild plots for prazosin were not significantly different from unity ($pK_B = 8.50$). WB4101 and HV723 competitively inhibited the noradrenaline-induced contraction with low pK_B values (approximately 8.30), irrespective of CEC-pretreatment. Methoxamine-induced contractions were competitively inhibited by prazosin, WB4101 and HV723 with low pK_B values similar to those obtained when noradrenaline was used as the agonist. These were not affected by CEC-pretreatment.

5 The affinity constant for noradrenaline ($pK_A = 6.40$) in CEC-untreated aorta was slightly greater than that obtained in CEC-pretreated aorta (5.78). On the other hand, methoxamine showed a similar affinity in CEC-untreated and pretreated aortae (pK_A = approximately 4.5).

6 Nifedipine (1 μM) slightly attenuated the contractile responses to noradrenaline and methoxamine in CEC-untreated and pretreated aortae, suggesting that nifedipine cannot discriminate between α_1-adrenoceptors involved in CEC-sensitive and -resistant contractions.

7 From these results it is suggested that in the rabbit thoracic aorta there are two distinct α_1-adrenoceptor subtypes (presumably α_{1B} and α_{1L} subtypes according to recently proposed subclassification), both of which are involved in noradrenaline-induced contraction. The α_{1L} subtype predominantly mediates the contraction induced by methoxamine.

Keywords: α_1-Adrenoceptor subtype; α_{1B}-adrenoceptor; α_{1L}-adrenoceptor; rabbit thoracic aorta; [^{3}H]-prazosin binding

Introduction

α_1-Adrenoceptors were originally subdivided into two subclasses (α_{1A} and α_{1B}) (Morrow & Creese, 1986; Minneman, 1988). However, subsequent functional and binding studies have suggested the possible existence of additional subtypes with different affinities for prazosin and other α_1-adrenoceptor antagonists (Flavahan & Vanhoutte, 1986; Mignot *et al.*, 1989; Muramatsu *et al.*, 1990a; 1991; Han & Minneman, 1991; Hiramatsu *et al.*, 1992). We previously subclassified α_1-adrenoceptors into three subgroups according to different affinities for prazosin and HV723 (α_{1H}: prazosin-high and HV723-low affinity site; α_{1L}: prazosin- and HV723-low affinity site; α_{1N}: prazosin-low but HV723-high affinity site) (Muramatsu *et al.*, 1990a) and subsequently suggested that the originally proposed α_{1A} and α_{1B} subtypes may be included in the α_{1H} group because of their high affinity for prazosin (Muramatsu *et al.*, 1991; Oshita *et al.*, 1991; Ohmura *et al.*, 1992).

Rabbit thoracic aorta is one of classical tissues used in pharmacological studies. Noradrenaline and other α_1-adrenoceptor agonists produce concentration-dependent contractions through α_1-adrenoceptors on the smooth muscle. Recently, we have found that the concentration-response curves for noradrenaline are displaced by prazosin in a manner which is not consistent with single competitive antagonism, suggesting the possible involvement of two distinct α_1-adrenoceptor subtypes in the contractile response to noradrenaline (Muramatsu *et al.*, 1990b). In the present study, we have characterized the α_1-adrenoceptor subtypes of rabbit thoracic aorta in more detail.

Methods

Binding experiments

The thoracic aortae were isolated from rabbits (2.5–3.5 kg) under pentobarbitone anaesthesia and homogenized in 20 vol

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of buffer (Tris HCl 50 mM, NaCl 100 mM, EDTA 2 mM, pH 7.4) with a polytron (setting 8, 15 s × 3) and centrifuged at 700 *g* for 10 min. The supernatant was filtered through four layers of cheese cloth and subjected to centrifugation at 10,000 *g* for 20 min; the supernatant was further centrifuged at 80,000 *g* for 30 min. The resulting pellet was suspended in assay buffer (Tris HCl 50 mM, EDTA 1 mM, pH 7.4), incubated at 37°C for 10 min, and again centrifuged under the same conditions mentioned above. The final pellet was resuspended in assay buffer and used for binding assay. The membranes were incubated with [^{3}H]-prazosin for 45 min at 30°C. Incubation volume was 1 ml (about 50 μg protein/tube). Reactions were terminated by rapid filtration through using a Brandel cell harvester with Whatman GF/C filters presoaked in 0.3% polyethylenimine for 5 min. The filters were then washed 4 times with 4 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and dried. The filter-bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined as binding in the presence of 10 μM phentolamine or 0.1 μM prazosin. Assays were conducted in duplicate.

CEC treatment Membrane preparations were incubated for 20 min at 37°C with 10 μM CEC and centrifuged at 80,000 *g* for 30 min. The pellet was washed once with assay buffer before the binding experiment.

Saturation binding data were analyzed by the weighted least-squares iterative curve fitting programme LIGAND (Munson & Rodbard, 1980). The data were first fitted to a one- and then a two-site model, and if the residual sums of squares were statistically less for a two-site fit of the data than for a one-site, as determined by an *F*-test comparison, then the two-site model was accepted. *P* values less than 0.05 were considered significant. Displacement binding data were analyzed by the EBDA programme (McPherson, 1985; Biosoft, Elsevier) when the slope factor was close to unity and IC_{50} values were converted to K_i values with the Cheng-Prusoff approximation (1973). However, when the slope factor seemed to deviate from unity, the LIGAND programme was used to determine which of one- or two-site model fitted. Proteins were assayed according to the method of Bradford with bovine serum albumin used as standard (Bradford, 1976).

Functional experiments

Rabbits were killed under pentobarbitone anaesthesia and the thoracic aorta was rapidly removed. The aorta was cleaned of adherent connective tissue and cut helically under a dissecting microscope. In order to avoid the possible involvement of endothelium-derived relaxing factor in the mechanical response (Furchgott, 1981), the endothelial cells were removed by rubbing them with filter paper (Muramatsu *et al.*, 1990b). The strip was mounted vertically in an organ bath containing 20 ml Krebs-Henseleit solution of the following composition (mM): NaCl 112, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11.5. Desmethylimipramine (0.1 μM), deoxycorticosterone (5 μM) and propranolol (3 μM) were added to the bathing solution to block neural and extraneural uptake of noradrenaline and to block β-adrenoceptors, respectively. The bath medium was maintained at 37°C, pH 7.4 and was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2. A resting tension of 1.5 g was applied and the responses were recorded isometrically through force-displacement transducers. All preparations were equilibrated for 90 min before the experiments were begun.

Concentration-response curves for noradrenaline and methoxamine were obtained by adding the drug directly to the bathing medium in cumulative concentrations. The curves were obtained 6 times in the same strip at 90 min intervals and the third concentration-response curve was used as control. There was little change in agonist sensitivity between the third and sixth concentration-response curves (Muramatsu *et al.*, 1990a). Preparations were treated with α-adrenoceptor antagonists for 30 min before, and during, the construction of concentration-response curves. With chloroethylclonidine (CEC) treatment, the preparation were treated once for 20 min with 10 μM CEC, then washed with the drug-free solution and two different concentrations of antagonist were subsequently tested in each preparation.

The pK_B values for α_1-adrenoceptor antagonists were estimated according to Arunlakshana & Schild (1959). Data were plotted as the −log antagonist concentration (M) vs the log (concentration ratio-1, CR-1), and pA_2 values along mean slope and 95% confidence limit (95% CL) calculated and straight lines were drawn by least square linear regression. When the straight line yielded a slope with unity, the pA_2 value estimated was represented as the pK_B (Arunlakshana & Schild, 1959). When a part of the data deviated from a straight line with a slope of unity (see Figure 3, solid circles at low concentrations of prazosin), the pK_B value was also determined for a single concentration of antagonist by the concentration method (Furchgott, 1972).

Dissociation constants (K_A) of adrenoceptor agonists were determined by comparing equieffective concentrations of the agonist under control conditions and after partial irreversible blockade of adrenoceptors with phenoxybenzamine (Furchgott, 1966). In this case, the preparations were treated with 20 or 70 nM phenoxybenzamine for 30 min and then washed 90 min before agonist responses were re-established.

Statistical analysis

Experimental values are given as the mean ± s.e.mean. Results were analysed by Student's *t* test and a probabilty of less than 0.05 was considered significant.

Drugs

The following drugs were used: [^{3}H]-prazosin (specific activity 87.0 Ci mmol^{-1}, NEN, Boston, U.S.A.), (−)-noradrenaline bitartrate, desipramine hydrochloride, nifedipine, prazosin hydrochloride (Sigma, St. Louis, U.S.A.), phentolamine mesylate (Ciba, Basel, Switzerland), WB4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride), chloroethylclonidine dihydrochloride (CEC) (Research Biochemicals Inc., Natick, U.S.A.), HV723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxyl)ethyl)-amino)-propyl) benzeneacetonitrile fumarate) (Hokuriku Seiyaku, Katsuyama, Japan), deoxycorticosterone acetate, (±)-propranolol hydrochloride, phenoxybenzamine hydrochloride (Nacalai, Kyoto, Japan) and methoxamine hydrochloride (Nippon-Shinyaku, Kyoto, Japan).

Results

Binding experiments

[^{3}H]-prazosin at concentrations ranging from 20–6000 pM was used to label α_1-adrenoceptors of rabbit thoracic aorta. The specific binding was approximately 90% of the total binding at 200 pM [^{3}H]-prazosin. Scatchard plots of the binding data were curvilinear, suggesting more than a single class of binding site. LIGAND analysis fitted the data to a two site model. The high affinity site showed a B_{max} of 79.2 ± 9.4 fmol mg^{-1} protein (R_{high}) and an equilibrium dissociation constant ($pK_{D\ high}$) of 9.94 ± 0.07, while the low affinity site showed R_{low} of 215.4 ± 54.1 fmol mg^{-1} protein and $pK_{D\ low}$ of 8.59 ± 0.29 ($n = 5$, Figure 1a and b).

In contrast, Scatchard plots of the data obtained from the membranes pretreated with 10 μM CEC were apparently linear, and the data best fitted to a one-site model. The pK_D value (9.07 ± 0.08) estimated was close to the value for the low affinity site in control membranes but the B_{max} value

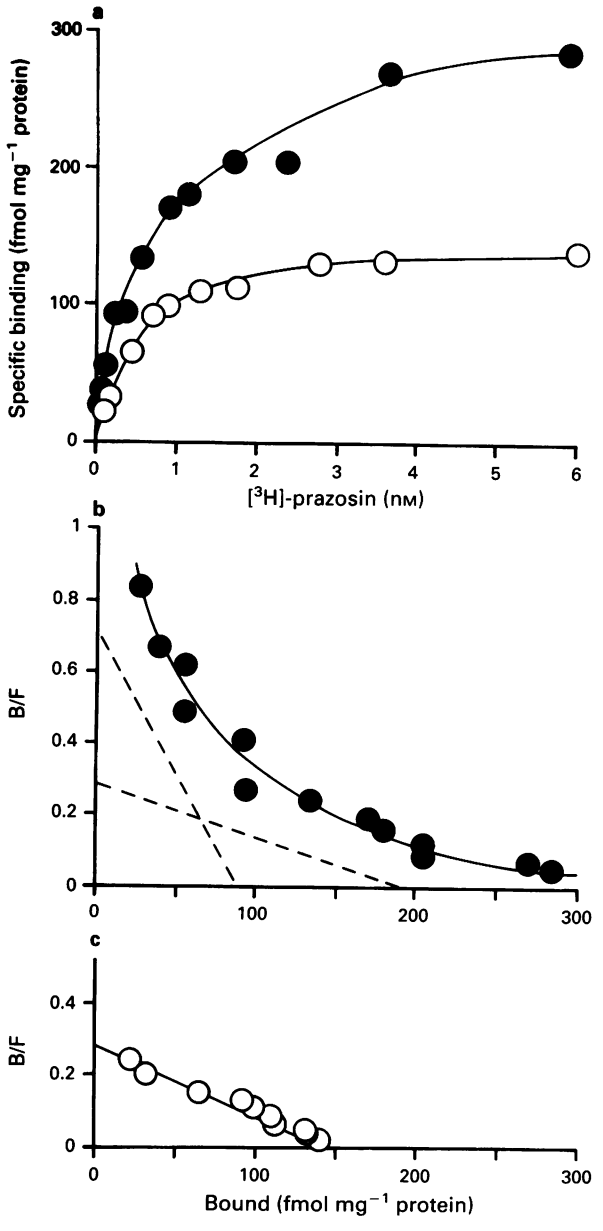

Figure 1 Saturation experiments of [^{3}H]-prazosin binding to rabbit thoracic aorta membranes. (a) Saturation curves of specific [^{3}H]-prazosin binding to chloroethylclonidine (CEC)-untreated (●) and CEC-pretreated membranes (○). (b) Scatchard analysis in CEC-untreated membranes. (c) Scatchard analysis in CEC-pretreated membranes. The data are obtained from a single experiment where each point is the mean of duplicate determinations.

(99.2 ± 15.6 fmol mg^{-1} protein, $n = 6$) was significantly lower than that of CEC-untreated membranes (Figure 1a and c).

In order to characterize the prazosin-high and low affinity sites, two kinds of displacement experiments were performed. Firstly, 200 pM [^{3}H]-prazosin binding sites in the CEC-untreated membranes were analyzed. Unlabelled prazosin displaced the binding monophasically with a high pK_i value (9.99). WB4101 and HV723 also antagonized the 200 pM [^{3}H]-prazosin binding. Computer analysis of the displacement curves gave a better fit to a one-site model, estimating more than 10 times lower affinities of WB4101 and HV723 as compared with that of prazosin (Figure 2a, Table 1).

Secondly, the low affinity sites for prazosin were characterized in the CEC-pretreated membranes, because prazosin-high affinity sites were found to be almost inactivated by

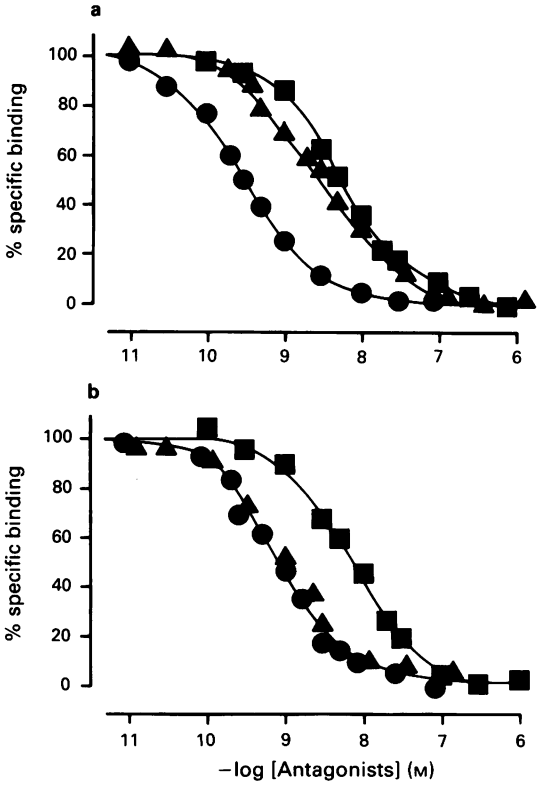

Figure 2 Displacement of [^{3}H]-prazosin binding from rabbit thoracic aorta membranes by prazosin (●), WB4101 (▲) and HV723 (■) in chloroethylclonidine (CEC)-untreated (a) and pretreated membranes (b). The concentrations of [^{3}H]-prazosin used were 200 pM in (a) and 1000 pM in (b).

Table 1 Inhibition of [^{3}H]-prazosin binding to α_1-adrenoceptors of rabbit thoracic aorta membranes by α-antagonists

	CEC-untreated			*CEC-pretreated*		
Drug	n	pK_i	*Slope factor*	n	pK_i	*Slope factor*
Prazosin	3	9.99 ± 0.05	1.05 ± 0.04	3	9.09 ± 0.03[a]	0.94 ± 0.03
WB4101	3	8.88 ± 0.05	0.88 ± 0.02	4	8.97 ± 0.06	0.92 ± 0.04
HV723	3	8.49 ± 0.10	1.07 ± 0.09	3	8.17 ± 0.11	1.03 ± 0.09
Noradrenaline	3	5.84 ± 0.06	0.91 ± 0.09	3	5.30 ± 0.05[a]	1.00 ± 0.04
Methoxamine	3	4.10 ± 0.06	0.86 ± 0.02	3	4.43 ± 0.08[a]	0.89 ± 0.06

Data shown are mean ± s.e.mean.
n: number of experiments.
[a] Significantly different from control (CEC-untreated) ($P < 0.05$).
CEC: membranes were pretreated with 10 μM chloroethylclonidine (CEC) for 20 min and then washed.
The concentrations of [^{3}H]-prazosin used were 200 pM in CEC-untreated membranes and 1,000 pM in CEC-pretreated membranes, respectively.

CEC pretreatment in saturation experiments. A higher concentration of [^{3}H]-prazosin (1000 pM) was used to label the low affinity sites. Unlabelled prazosin also displaced the [^{3}H]-prazosin binding in a monophasic manner ($pK_i = 9.09 \pm 0.03$). HV723 displaced 1000 pM [^{3}H]-prazosin binding and the pK_i value was similar to that obtained in CEC-untreated membranes (Figure 2b, Table 1).

Displacement profiles for noradrenaline and methoxamine at prazosin-high and low affinity sites were also examined in CEC-untreated and pretreated membranes. These agonists inhibited the binding of [^{3}H]-prazosin. The pK_i value (5.84) for noradrenaline in CEC-untreated membranes was approximately 3 times higher than that (5.30) in CEC-pretreated membranes. Conversely, the affinity for methoxamine was only slightly higher in the CEC-pretreated membranes (Table 1).

Functional study

Noradrenaline and methoxamine produced a concentration-dependent contraction in rabbit isolated thoracic aorta. Prazosin antagonized the contractions induced by noradrenaline and produced a rightward shift of the concentration-response curves. The slope of the Schild plot for prazosin was significantly different from unity (slope = 0.723, 95% CL = 0.668–0.779), so the occurrence of two different affinity sites was suggested (Figure 3 and Table 2). However, the concentration-response curve for methoxamine was inhibited by prazosin in a simple competitive manner. WB4101 and HV723 also antagonized the contractions induced by both agonists with a Schild slope close to unity. Thus, single affinity constants for both antagonists were estimated (Table 2).

We have previously shown that CEC selectively masked the contractions mediated through high affinity sites for prazosin (Muramatsu *et al.*, 1990b). In the present study, pretreatment with CEC (10 μM) also shifted the concentration-response curve for noradrenaline to the right (pD_2; from 7.18 ± 0.04 to 6.88 ± 0.04, $n = 14$) without affecting the maximal contraction. On the other hand, the concentration-response curve for methoxamine was not significantly affected by CEC pretreatment (pD_2; 5.79 ± 0.04 and 5.75 ± 0.05 without and with CEC pretreatment, respectively, $n = 14$). All the antagonists tested blocked competitively the noradrenaline- and methoxamine-induced contractions in CEC-pretreated aorta (Table 2). The pK_B value for prazosin was similar to the value for low affinity site obtained in CEC-untreated aorta. The pK_B values for WB4101 and HV723 were not significantly different from the pK_B values in the CEC-untreated aorta.

To evaluate the affinity for noradrenaline and methoxamine in CEC-pretreated and untreated aortae, the effect of phenoxybenzamine was examined. Pretreatment with phenoxybenzamine (20 or 70 nM) produced an irreversible attenuation of contractile responses to noradrenaline or methoxamine. The calculated dissociation constant (pK_A value) for noradrenaline in CEC-untreated aorta was slightly higher than that in CEC-pretreated aorta. The pK_A values for methoxamine were not affected by CEC-pretreatment (Table 3).

Finally, effects of nifedipine were examined. Nifedipine (1 μM) slightly but significantly attenuated the contractile responses to noradrenaline or methoxamine both in CEC-untreated and pretreated aortae (Figure 4).

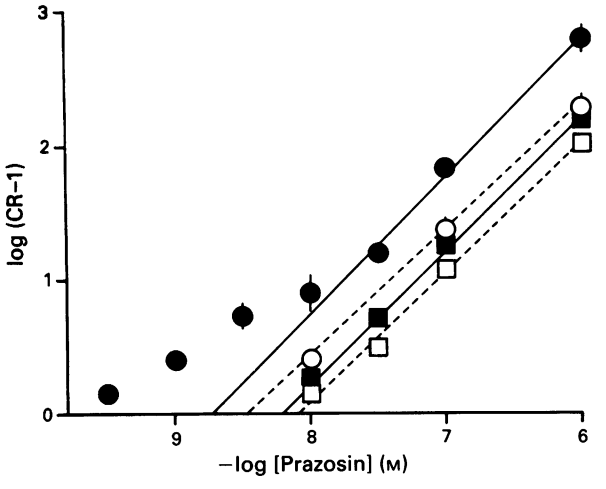

Figure 3 Schild plots for prazosin against contractile responses to noradrenaline (●, ○) or methoxamine (■, □). Filled symbols: chloroethylclonidine (CEC)-untreated aorta; open symbols: CEC-pretreated aorta.

Table 3 The dissociation constants (pK_A) of noradrenaline and methoxamine in rabbit thoracic aorta

	pK_A	
Treatment	*Noradrenaline*	*Methoxamine*
None	6.40 ± 0.11	4.48 ± 0.16
CEC	5.78 ± 0.10[a]	4.58 ± 0.13

Data shown are mean ± s.e.mean ($n = 8–10$).
CEC: 10 μM chloroethylclonidine.
The pK_A values were obtained by the method of Furchgott (1966) using 20 or 70 nM phenoxybenzamine.
[a] Significantly different from control (no treatment) ($P<0.05$).

Table 2 Antagonism by prazosin, WB4101 and HV723 of the contractile responses to noradrenaline and methoxamine in rabbit thoracic aorta

	pK_B (Slope, 95% CL)	
Antagonist	*No treatment*	*After CEC*
(I) Noradrenaline-induced contraction		
Prazosin	9.71 ± 0.08[a]	
WB4101	8.74 ± 0.15 (1.02, 0.85–1.19)	8.50 ± 0.09 (0.90, 0.79–1.01)
HV723	8.27 ± 0.05 (0.99, 0.92–1.06)	8.24 ± 0.07 (0.92, 0.83–1.02)
	8.49 ± 0.06 (0.95, 0.88–1.03)	8.33 ± 0.07 (0.92, 0.84–1.01)
(II) Methoxamine-induced contraction		
Prazosin	8.22 ± 0.04 (1.00, 0.93–1.06)	8.20 ± 0.03 (0.96, 0.91–1.01)
WB4101	8.49 ± 0.07 (0.98, 0.90–1.06)	8.51 ± 0.08 (0.98, 0.90–1.07)
HV723	8.16 ± 0.09 (1.07, 0.92–1.22)	8.36 ± 0.08 (0.94, 0.85–1.03)

[a] The pK_B value was estimated from the inhibitory effect of 300 pM prazosin. The other data were obtained from Schild plots.
CEC: 10 μM chloroethylclonidine.

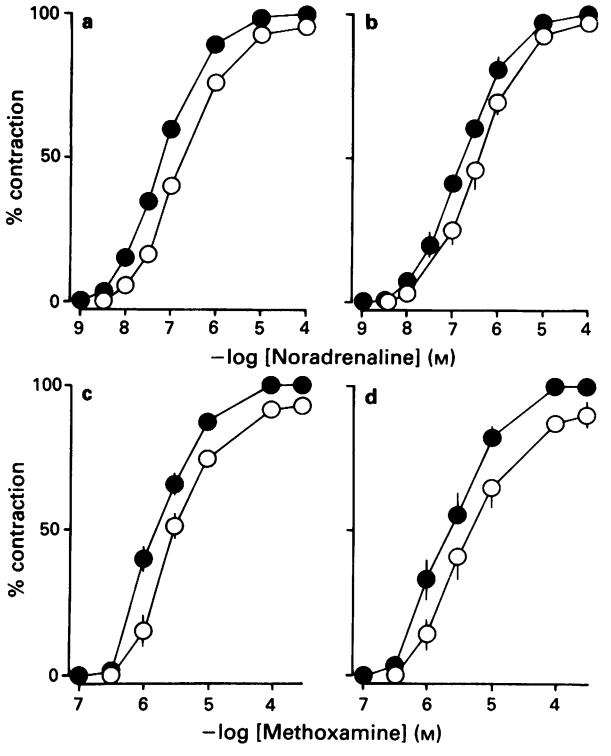

Figure 4 Effects of nifedipine on the concentration-response curves for noradrenaline (a, b) or methoxamine (c, d). (a, c) Chloroethylclonidine (CEC)-untreated aorta; (b, d): CEC-pretreated aorta. (●): control, (○): 1 μM nifedipine. The data shown are means ± s.e.mean of 5–6 experiments. All the contractile responses to noradrenaline or methoxamine were slightly but significantly ($P<0.05$) attenuated by nifedipine even though symbols overlap each other.

Discussion

The presence of two distinct α_1-adrenoceptors has been reported in the rabbit thoracic aorta (Babich *et al.*, 1987; Piascik *et al.*, 1988; Muramatsu *et al.*, 1990b). The present binding study reveals that α_1-adrenoceptors are clearly discriminated by prazosin; i.e., prazosin-high and low affinity sites. According to the α_{1H}, α_{1L}, α_{1N} subclassification (Muramatsu *et al.*, 1990a), both the sites appear to respectively correspond to α_{1H} and α_{1L} subtypes, as suggested previously (Muramatsu *et al.*, 1990b).

α_1-Adrenoceptors with high affinity for prazosin have been further subdivided into four subclasses (α_{1A}, α_{1B}, α_{1C} and α_{1D}) in binding and molecular biological studies (Han & Minneman, 1991; Lomasney *et al.*, 1991; Perez *et al.*, 1991; Hiramatsu *et al.*, 1992). We recently proposed the possibility that such subtypes may be included in the α_{1H} group designated in the α_{1H}, α_{1L}, α_{1N} subclassification (Muramatsu *et al.*, 1991; Oshita *et al.*, 1991; Ohmura *et al.*, 1992). Only the α_{1B} subtype among α_{1H} group shows low affinity for WB4101 and can be markedly inactivated by an alkylating agent, CEC. In this study, we determined which of the subtypes corresponds to the prazosin-high affinity site (α_{1H}) of rabbit thoracic aorta. The results obtained in binding experiments clearly indicate that the prazosin-high affinity site of rabbit thoracic aorta is a low affinity site for WB4101 and is markedly inactivated by CEC, corresponding to the features of an α_{1B}-adrenoceptor subtype. Expression of α_{1B}, but not α_{1A} and α_{1C} subtypes, in the rabbit thoracic aorta was also demonstrated by northern blot analysis (Schwinn *et al.*, 1991).

The prazosin-high and low affinity sites are also found from the antagonistic manner of prazosin against the contractile response to noradrenaline. However, WB4101 and HV723 cannot discriminate between the sites, resulting in a simple competition with a low affinity for each drug. Pretreatment with CEC abolished the inhibitory effect of low concentrations of prazosin but did not affect the inhibitory potency of WB4101 or HV723. These results support our previous view that noradrenaline-induced contraction is produced through two distinct α_1-adrenoceptor populations (presumably α_{1H} and α_{1L}) in the rabbit thoracic aorta (Muramatsu *et al.*, 1990b), and further suggest that the prazosin-high affinity site (α_{1H}) is characterized as an α_{1B} subtype.

In contrast to the response to noradrenaline, prazosin inhibited the contractile responses to methoxamine in a competitive manner and pretreatment with CEC failed to affect the response to methoxamine. Since similar and low pK_B values were estimated for prazosin, WB4101 or HV723 irrespective of CEC pretreatment, the contractile response to methoxamine appears to be produced through a single subtype (presumably α_{1L}). Alternatively, even though two different subtypes (α_{1B} and α_{1L}) are involved in the contractions induced by methoxamine, the α_{1L} subtype would play a more predominant role in the response to methoxamine than the α_{1B} subtype, because either prazosin at concentrations low enough to bind to the α_{1B} subtype or CEC to inactivate the α_{1B} subtype had no effect on the contractile response to methoxamine.

We compared the affinities of α_{1B} and α_{1L} subtypes for both agonists. Binding data clearly show that the α_{1B} subtype is slighly but significantly more sensitive to noradrenaline than the α_{1L} subtype, whereas methoxamine tends to reverse the selectivity slightly. In the functional experiments also, similar results were obtained for the dissociation constant (pK_A) for noradrenaline, but there was no significant difference in the values for methoxamine between CEC-untreated and pretreated aortae. This, together with the binding data mentioned above, suggests the predominant involvement of the α_{1L} adrenoceptor subtype in the contractile response to methoxamine compared with the response to noradrenaline.

The dissociation constants for α_1-adrenoceptor agonists and antagonists estimated in the functional experiments may be approximate values because, control concentration-response curves obtained in the absence of α_1-adrenoceptor antagonists might be caused through activation of two receptor subtypes, while the curves obtained after treatment with antagonist may be mainly produced through a single subtype. The contribution of each subtype to the total response may change depending on experimental conditions.

CEC was originally reported to produce a selective inactivation of the α_{1B} subtype (Minneman *et al.*, 1988). Although almost complete inactivation of the prazosin-high affinity site (α_{1B} subtype) was indeed produced by 10 μM CEC in the present study, [^{3}H]-prazosin binding to the low affinity site (α_{1L} subtype) was also in part inhibited. Such partial inactivation or inhibition of [^{3}H]-prazosin binding has been reported in other subtypes including α_{1A} and α_{1C} subtypes (Schwinn *et al.*, 1991; Oshita *et al.*, 1991; Ohmura *et al.*, 1992), indicating a relatively low selectivity of CEC to α_1-adrenoceptor subtypes.

Even under conditions where the α_{1L} subtype was in part inactivated by CEC, noradrenaline and methoxamine produced a maximum contraction in the rabbit thoracic aorta. This indicates that both drugs can act as full agonists for the α_{1L} subtype, and further suggests that there are spare receptors in the α_{1L} subtype-mediated contractions in the rabbit thoracic aorta. Higher density of α_{1L} than α_{1B} subtype and higher value of pD_2 than pK_A for noradrenaline or methoxamine would support this suggestion.

Han *et al.* (1987) have proposed that the α_{1A} receptor subtype selectively mediates the influx of extracellular Ca^{2+}, whereas the α_{1B} subtype controls the release of stored intracellular Ca^{2+} by an inositol phosphate mechanism. Following this original proposal, Suzuki *et al.* (1990) suggested that phenylephrine-induced contraction of the rabbit thoracic aorta may be mediated through α_{1A} and α_{1B} subtypes. However, mRNA for the α_{1A} subtype was not detected in the

rabbit aorta (Schwinn *et al.*, 1991) and WB4101-high affinity sites were not found in the present binding and functional studies. Furthermore, the contractile responses induced by noradrenaline or methoxamine in CEC-pretreated aorta were not abolished by nifedipine. Although nifedipine has been demonstrated to produce a non-selective inhibition of the contractile responses mediated through different α_1-adrenoceptor subtypes (Muramatsu *et al.*, 1990a; 1991; Ohmura *et al.*, 1992), the slight inhibition by nifedipine observed in the present study does not suggest the possible occurrence of the α_{1A} subtype in the rabbit thoracic aorta.

In conclusion, the present study shows the occurrence of two distinct α_1-adrenoceptor subtypes (prazosin-high and low affinity sites) in the rabbit thoracic aorta. Although the prazosin-high affinity site was previously designated as α_{1H} (Muramatsu *et al.*, 1990b), the pharmacological features obtained indicate that the site corresponds to the α_{1B} subtype (a member of α_{1H} group) according to the recently conciliated subclassification, while the prazosin-low affinity site is of the α_{1L} subtype which is clearly different from the other subtypes in its pharmacological features. It is likely that noradrenaline-induced contraction is mediated through both the α_{1B} and α_{1L} subtypes, whereas the response to methoxamine is predominantly caused through the α_{1L} subtype.

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