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European Journal of Pharmacology 374 (1999) 229–240

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Analysis of α_1-adrenoceptor subtypes in rabbit aorta and arteries: regional difference and co-existence

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Received 14 January 1999; received in revised form 29 April 1999; accepted 5 May 1999

Abstract

This study was done to determine the α_1-adrenoceptor subtypes and to characterize the functional role of α_{1D}-adrenoceptors in the following rabbit arteries: thoracic and abdominal aorta, mesenteric, renal and iliac arteries. In all arteries, selective α_{1D}-adrenoceptor antagonist BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspirol(4,5) decane-7,9-dione dihydrochloride) dose dependently shifted the concentration–response curves for norepinephrine to the right. Schild plots of the results obtained from the inhibition by BMY 7378 for norepinephrine yielded a straight line with a slope of unity in thoracic (pA_2 6.54 ± 0.02) and abdominal (pA_2 6.73 ± 0.03) aorta. Slopes of Schild plots obtained from the inhibition by BMY 7378 for norepinephrine were significantly different from unity in mesenteric, renal and iliac arteries. Slopes of Schild plots for BMY 7378 were not different from unity in chloroethylclonidine-treated thoracic (pA_2 6.49 ± 0.14) and abdominal (pA_2 6.61 ± 0.11) aorta. Slopes of Schild plots for BMY 7378 were significantly different from unity in chloroethylclonidine-treated mesenteric, renal and iliac arteries. On the other hand, in Ca^{2+}-free physiological saline solution (Ca^{2+}-free PSS) slopes obtained from Schild plots for BMY 7378 were not different from unity in thoracic (pA_2 6.41 ± 0.09) and abdominal (pA_2 6.28 ± 0.07) aorta and mesenteric (pA_2 6.55 ± 0.06), renal (pA_2 6.24 ± 0.10) and iliac (pA_2 6.64 ± 0.13) arteries. BMY 7378 inhibited [^{3}H]prazosin binding to thoracic (pK_i 6.44 ± 0.08) and abdominal (pK_i 6.59 ± 0.02) aorta with low potency, and mesenteric ($pK_{i\ High}$ 8.66 ± 0.28, $pK_{i\ Low}$ 6.34 ± 0.14), renal ($pK_{i\ High}$ 8.71 ± 0.33, $pK_{i\ Low}$ 6.45 ± 0.03) and iliac artery ($pK_{i\ High}$ 8.60 ± 0.24, $pK_{i\ Low}$ 6.56 ± 0.13). These results suggest that α_{1D}-adrenoceptors play a significant role for contractile responses in renal and iliac artery, but play virtually no role in thoracic and abdominal aorta and that an α_1-adrenoceptor subtype, which is pharmacologically distinguishable from the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtype, may co-exist in mesenteric artery. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: α_1-Adrenoceptor; BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspirol(4,5) decane-7,9-dione dihydrochloride); Regional difference; Artery, Rabbit

1. Introduction

α_1-adrenoceptors have been classified according to their pharmacological properties into α_{1A}- and α_{1B}-adrenoceptor subtypes (Morrow and Creese, 1986; Han et al., 1987; Minneman et al., 1988). Recently α_1-adrenoceptors were pharmacologically divided into three major subtypes, α_{1A}, α_{1B} and α_{1D}, based on differences in their affinities for selective competitive antagonists and an irreversible antagonist. The α_{1A}-adrenoceptor subtype has a high affinity for antagonists, WB 4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane), 5-methylurapidil, and (+)-niguldipine (Boer et al., 1989; Gross et al., 1989; Tsujimoto et al., 1989), while the α_{1B}-adrenoceptor subtype is irreversibly inactivated by an alkylating agent, chloroethylclonidine (Han et al., 1987). These antagonists have been extensively used for the pharmacological characterization of α_{1A}- and α_{1B}-adrenoceptor subtypes. We had reported that α_{1A}-adrenoceptor subtypes in rabbit thoracic aorta account for 20% of α_1-adrenoceptors, approximately and there are two α_1-adrenoceptor subtypes α_{1A} and α_{1B}, at least in rabbit thoracic aorta and iliac artery (Takayanagi et al., 1991a; Satoh et al., 1992a,b). The α_{1D}-adrenoceptor subtype has a high affinity for the antagonist, BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-

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PII: S0014-2999(99)00340-4

piperazinyl)ethyl)-8-azaspirol(4,5) decane-7,9-dione dihydrochloride) (Saussy et al., 1994), which is a pharmacological probe to study α_{1D}-adrenoceptor function. The α_{1D}-adrenoceptor subtype exists in various tissues (Price et al., 1994; Scofield et al., 1995; Wada et al., 1996; Nasu et al., 1998) and also plays an important role in muscle contraction in rat aorta (Kenny et al., 1995; Testa et al., 1995). Molecular biological techniques have demonstrated the existence of distinct genes coding for these different α_1-adrenoceptor subtypes, α_{1a}, α_{1b} and α_{1d}, in various

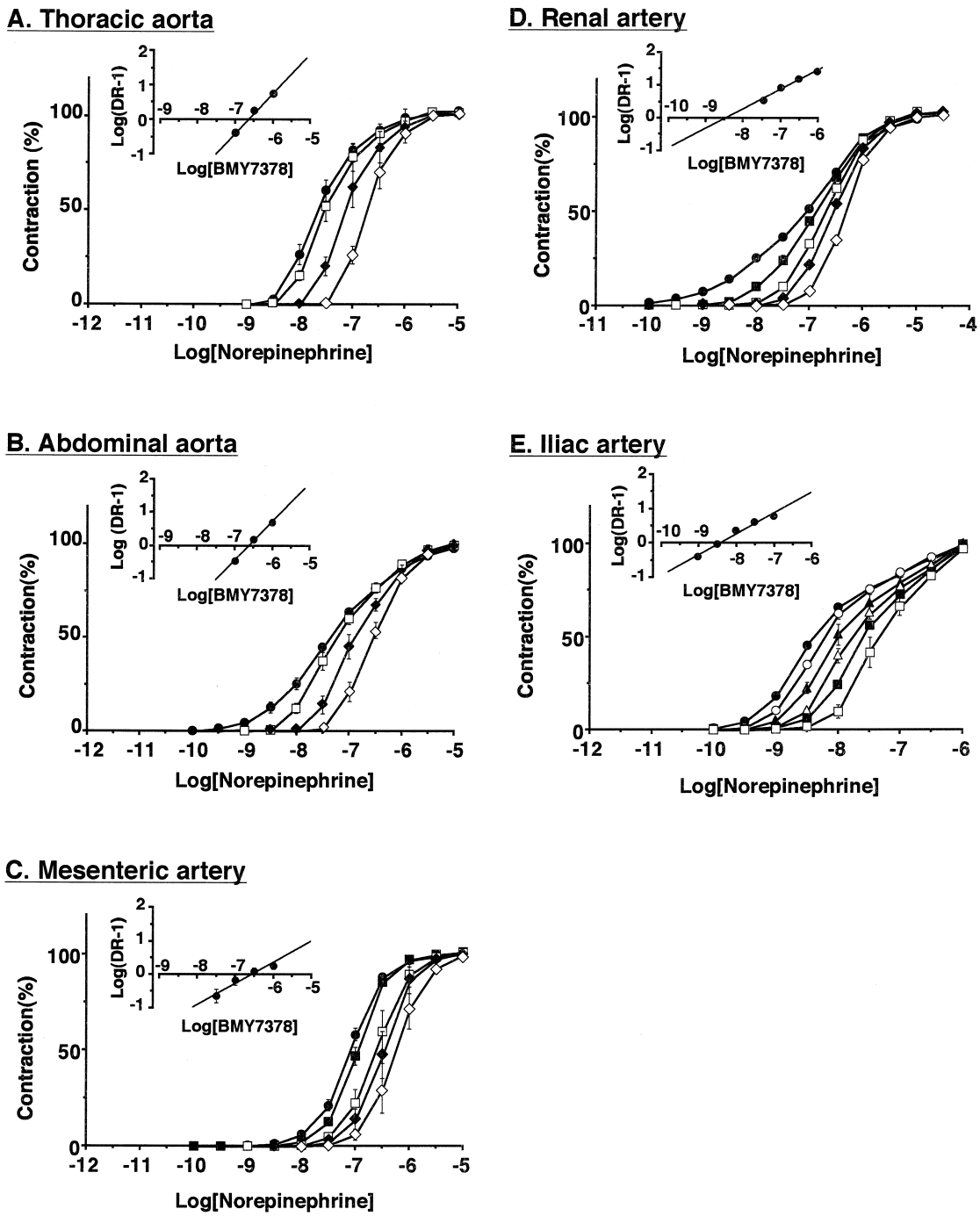

Fig. 1. Effect of BMY 7378 on norepinephrine-induced contraction and Schild plots for antagonism between norepinephrine and this antagonist in five arteries of rabbit. (A) Thoracic aorta; (B) abdominal aorta; (C) mesenteric artery; (D) renal artery; (E) iliac artery. Ordinate: contraction (%) which is expressed as a percentage of the contractile response to norepinephrine (3×10^{-6} M). Abscissa: logarithm of norepinephrine concentration (M). ●, norepinephrine alone; ○, 10^{-9} M BMY 7378; ▲, 3×10^{-9} M BMY 7378; △, 10^{-8} M BMY 7378; ■, 3×10^{-8} M BMY 7378; □, 10^{-7} M BMY 7378; ◆, 3×10^{-7} M BMY 7378; ◇, 10^{-6} M BMY 7378. Inset, Schild plots. Ordinate: logarithm of equieffective concentration ratio (DR) of norepinephrine minus 1. Abscissa: logarithm of molar concentration of BMY 7378. In renal artery, Schild regression analysis was done at 20% contraction. Each value is presented as the mean ± S.E. (bar).

animals (Cotecchia et al., 1988; Voigt et al., 1990; Bylund et al., 1994; Burt et al., 1995) and in humans (Bruno et al., 1991; Ramarao et al., 1992; Hirasawa et al., 1993). The three α_1-adrenoceptor subtypes are designated α_{1A}, α_{1B} and α_{1D} for the native receptors and α_{1a}, α_{1b} and α_{1d} for the cloned receptors (Hieble et al., 1995). In addition, the existence of a fourth α_1-adrenoceptor subtype, the putative α_{1L}-adrenoceptor subtype (Muramatsu et al., 1990), has been proposed on the basis of functional studies.

Regional differences in the sensitivity of rabbit (Bevan et al., 1986; Oriowo et al., 1992; Satoh et al., 1998) and canine (Shoji et al., 1983; Griendling et al., 1984; Takayanagi et al., 1987, 1988) isolated blood vessels to norepinephrine have been detected. Bevan et al. (Bevan et al., 1986; Oriowo et al., 1987) determined norepinephrine sensitivity and affinity of norepinephrine to α_1-adrenoceptors in mechanical experiments, and they concluded that agonist affinity was the primary determinant of sensitivity to norepinephrine and that this was a locally regulated characteristic which might account for regional sensitivity changes. Takayanagi et al. (1991a) and Satoh et al. (1992a,b) showed that the thoracic aorta and iliac arteries of rabbit contain both α_{1A}- and α_{1B}-adrenoceptor subtypes. Each receptor subtype has a distinct role: α_{1A}-adrenoceptor subtypes cause a tonic response predominantly dependent on the influx of extracellular Ca^{2+}, whereas α_{1B}-adrenoceptor subtypes cause a phasic response that is predominantly independent of extracellular Ca^{2+} since it is stimulated by intracellular Ca^{2+} mobilization by phosphatidylinositol hydrolysis (Suzuki et al., 1990). Each subtype has an important role in the contraction of vascular smooth muscle. Further Satoh et al. (1998) reported that the regional differences in potency (pD_2 value) of the α_1-adrenoceptor agonist, norepinephrine, were due to the differences in population and density of α_1-adrenoceptor subtypes (α_{1A} and α_{1B}), and noted the possibility of other subtypes (α_{1D}) co-existing in the arteries of rabbit. To determine the regional differences and the co-existence of α_{1D}-adrenoceptor subtypes in the present study, we characterized pharmacologically the functional roles of the α_{1D}-adrenoceptor subtype in the contraction of vascular smooth muscle, using a selective α_{1D}-adrenoceptor antagonist, BMY 7378. We also conducted BMY 7378 binding displacement studies using [^{3}H]prazosin and blood vessels from five different regions (thoracic and abdominal aorta, mesenteric, renal and iliac arteries) of rabbit.

2. Methods

2.1. General

Male albino rabbits weighing 2.0–3.0 kg were anesthetized with an intravenous injection of pentobarbital sodium (50 mg/kg) and killed by bleeding from the carotid arteries. Thoracic and abdominal aorta and mesenteric, renal and iliac arteries were quickly removed and dissected free of excess fat and connective tissue in oxygenated physiological saline solution (PSS) of the following composition (in millimoles): NaCl, 118; $MgCl_2$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25 and glucose, 11.0 dissolved in distilled water (pH = 7.4 at 37°C). Ca^{2+}-free PSS was prepared without $CaCl_2$ and with 2 mM ethylene glycol bis (β-aminoethylether) *N,N′*-tetraacetic acid (EGTA). The solution contained propranolol (10^{-6} M), yohimbine (3×10^{-7} M), desmethylimipramine (10^{-7} M), and normetanephrine (10^{-6} M) to block β-adrenoceptors and α_2-adrenoceptors and to inhibit neural and non-neural uptake of catecholamines, respectively. The arteries were cut into helical strips about 10 mm in length and 2 mm in width. In order to avoid the possible involvement of endothelium-derived relaxing factor in the mechanical response, the endothelial cells were removed by gently rubbing with filter paper, and the functional loss of endothelial cells was confirmed by the loss of the relaxation response to acetylcholine (10^{-6} M) in norepinephrine-precontracted aorta. Effects of chloroethylclonidine, an irreversible antagonist of α_1-adrenoceptors, were tested. After determination of the control concentration-response curves for norepinephrine, the strips were treated with chloroethylclonidine (10^{-4} M) for a total of 60 min; following its initial application, the antagonist was renewed every 10 min at the same time. The concentration–response curves for norepinephrine were made after sufficient repetitions had been done to confirm that constant curves had been obtained.

2.2. Mechanical responses

The strips were suspended in a 20-ml organ bath filled with PSS gassed with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°C. The response to an agonist was isometrically recorded under a resting tension of 1 g for thoracic and abdominal strips, 0.5 g for mesenteric, renal and iliac arteries. The strips were allowed to equilibrate for 90 min, were then contracted with norepinephrine (10^{-7} M), and allowed to equilibrate for 30 min after washout.

Table 1
pA_2 Values for BMY 7378 against norepinephrine and slope of Schild plot for antagonism between norepinephrine and BMY 7378 in rabbit arteries. Each value is presented as a mean ± S.E. of four separate experiments. In renal artery Schild regression analysis was done at 20% contraction

Arteries	*n*	pA_2 Value	Slope
Thoracic aorta	4	6.54 ± 0.02	1.05 ± 0.02
Abdominal aorta	4	6.73 ± 0.03	0.97 ± 0.03
Mesenteric artery	4	6.56 ± 0.13[A]	0.57 ± 0.05[a]
Renal artery	4	8.50 ± 0.24[A]	0.61 ± 0.10[a]
Iliac artery	4	8.41 ± 0.09[A]	0.59 ± 0.02[a]

[A] Intercept of the Schild plot.
[a] Significant difference from unity ($P < 0.05$).

This was repeated until two successive contractions of approximately equal size had been obtained. Competitive antagonistic activities were expressed as pA_2 values (negative logarithms of the dissociation constant). After determination of control concentration–response curves, the strips were equilibrated with a competitive antagonist for 10 min. Concentration–response curves were then obtained in the presence of the antagonist and the procedure was repeated with a high (either 3- or 10-fold increase) concentration of the antagonist and the same preparation.

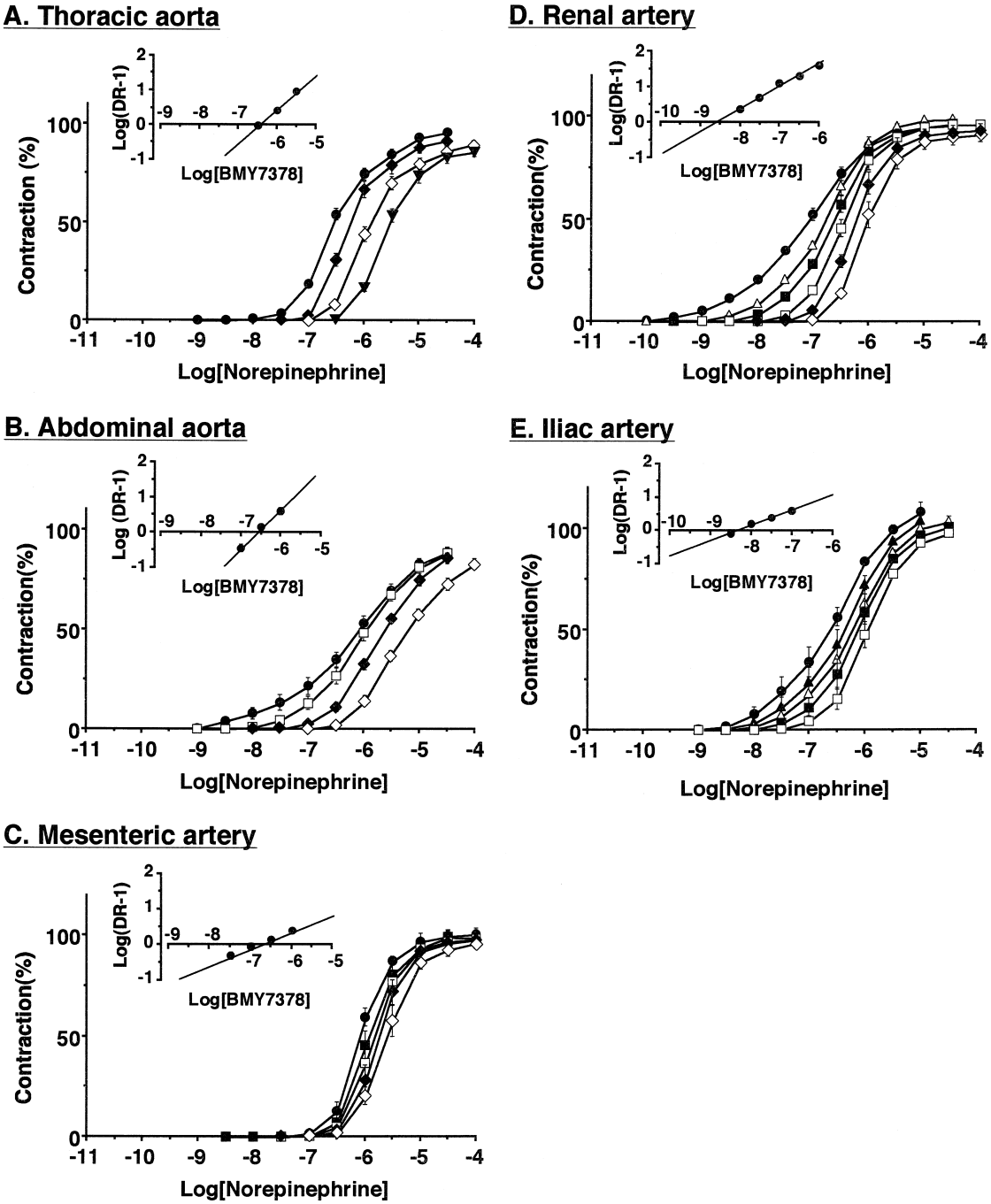

Fig. 2. Effect of BMY 7378 on norepinephrine-induced contraction and Schild plots for antagonism between norepinephrine and this antagonist in chloroethylclonidine-pretreated five arteries of rabbit. (A) Thoracic aorta; (B) abdominal aorta; (C) mesenteric artery; (D) renal artery; (E) iliac artery. Ordinate: Contraction (%) which is expressed as a percentage of the contractile response to norepinephrine (3×10^{-6} M). Abscissa: logarithm of norepinephrine concentration (M). ●, norepinephrine alone; ▲, 3×10^{-9} M BMY 7378; △, 10^{-8} M BMY 7378; ■, 3×10^{-8} M BMY 7378; □, 10^{-7} M BMY 7378; ◆, 3×10^{-7} M BMY 7378; ◇, 10^{-6} M BMY 7378; ▼, 3×10^{-6} M BMY 7378; ▽, 10^{-5} M BMY 7378. Inset, Schild plots. Ordinate: logarithm of equieffective concentration ratio (DR) of norepinephrine minus 1. Abscissa: logarithm of molar concentration of BMY 7378. In renal artery Schild regression analysis was done at 20% contraction. Each value is presented as the mean ± S.E. (bar). Tissues were pretreated with chloroethylclonidine (10^{-4} M) for 60 min.

After determination of the control, concentration–response curves for norepinephrine were made. The curves were nearly superimposable and changes in sensitivity, sensitization, or desensitization were minimal. The pA_2 values were calculated according to the method of Arunlakshana and Schild (1959). In some experiments, concentration–response curves for norepinephrine were obtained in Ca^{2+}-free PSS. Generally, norepinephrine was added after exposure of the muscle strips to Ca^{2+}-free PSS for 5 min.

2.3. Membrane preparation

Tissues were carefully cleaned of the adventitia and adherent connective tissues and endothelium. The tissues were homogenized with a Teflon-glass homogenizer in 100 vol. of ice-cold buffer (Tris–HCl 5 mM, sucrose 0.25 M, pH 7.4) and using a Polytron (setting 8, 15 s × 2). The homogenate was filtered through four layers of cheesecloth and centrifuged at $5000 \times g$ for 20 min at 4°C. The supernatant was centrifuged at $100{,}000 \times g$ for 60 min at 4°C. The pellet was resuspended in the same volume of buffer (Tris–HCl 50 mM, EGTA 1 mM, trypsin inhibitor and leupeptin 1 mg/l, pH 7.4), incubated for 10 min at 37°C, and centrifuged again as described above. All membrane preparation procedures used ice-cold buffers. The final pellet was resuspended in assay buffer (Tris–HCl 50 mM, pH 7.4) to yield 1.0–1.5 mg protein ml^{-1} and was used for the binding assay. Proteins were determined by the dye-binding method with bovine serum albumin as the standard.

2.4. [^{3}H]prazosin binding

Membranes prepared from rabbit thoracic and abdominal aorta, mesenteric, renal and iliac arteries were incubated with 0.6 nM [^{3}H]prazosin in the absence and the presence of increasing concentrations of BMY 7378 in 50 mM Tris buffer for 60 min at room temperature according to the procedure described by Takayanagi et al. (1991b) and Deng et al. (1996). The reaction was terminated by rapid filtration (Cell Harvester, Brandel, Gaithersburg, MD) through Whatman GF/B glass fiber filters, and the filters were rinsed 3 times with 4 ml of ice-cold buffer. Membrane-bound radioactivity was extracted from the filters overnight in scintillation fluid and the radioactivity was determined in a liquid scintillation counter. Specific [^{3}H]prazosin binding was determined experimentally from the difference between counts in the absence and presence of 10 μM phentolamine. All assays were conducted in duplicate. The apparent dissociation constant (K_D) and B_{max} for [^{3}H]prazosin was estimated by Scatchard analysis of the saturation data over a concentration range of 0.01 to 1.0 nM (Scatchard, 1949). The ability of antagonists to inhibit specific [^{3}H]prazosin binding was estimated from the IC_{50} value which was the molar concentration of unlabeled drug necessary to displace 50% of the specific binding (determined by log probit analysis). The value of the inhibition constant K_i was calculated from the equation $K_i = IC_{50}/(1 + L/K_D)$, where L equals the concentration of [^{3}H]prazosin (Cheng and Prusoff, 1973). The Hill coefficient for the competition data of [^{3}H]prazosin and inhibition by a drug was obtained by pseudo-Hill plot analysis. Data obtained from competition studies were analyzed by the weighted least-squares iterative curve-fitting program, LIGAND (Munson and 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.

2.5. Statistics

Numerical results are expressed as means ± S.E., and statistical significance was calculated with Student's t-test or Duncan's new multiple range test. A P value less than 0.05 was considered to indicate a significant difference.

2.6. Drugs

The following drugs were used: (−)-norepinephrine bitartrate (Wako-Junyaku, Osaka, Japan); 5-methyl-urapidil, BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)-ethyl)-8-azaspiro(4,5) decane-7,9-dione dihydrochloride) and chloroethylclonidine dihydrochloride (Research Biochemicals, Natick, MA); desmethylimipramine hydrochloride, (±)-normetanephrine hydrochloride, (±)-propranolol hydrochloride, yohimbine hydrochloride and bovine serum albumin (Sigma, St. Louis, MO), ethylene glycol bis (β-aminoethylether) N,N'-tetraacetic acid (EGTA) (Dojindo Laboratories, Kumamoto, Japan) all in powder form, phentolamine hydrochloride (Ciba-Geigy, Basel, Switzerland) and [^{3}H]prazosin (specific activity 82 Ci/mmol, NEN, Boston, USA). Other chemicals used were of analytical grade.

Table 2
pA_2 Values for BMY 7378 against norepinephrine and slope of Schild plot for antagonism between norepinephrine and BMY 7378 in chloroethylclonidine-treated rabbit arteries. Each value is presented as a mean ± S.E. of four separate experiments. Tissues are pretreated with chloroethylclonidine (10^{-4} M) for 60 min. In renal artery Schild regression analysis was done at 20% contraction

Arteries	n	pA_2 Value	Slope
Thoracic aorta	4	6.49 ± 0.14	0.97 ± 0.02
Abdominal aorta	4	6.61 ± 0.11	1.03 ± 0.03
Mesenteric artery	4	6.53 ± 0.16[A]	0.47 ± 0.07[a]
Renal artery	4	8.61 ± 0.11[A]	0.55 ± 0.32[a]
Iliac artery	4	8.41 ± 0.04[A]	0.37 ± 0.06[a]

[A] Intercept of the Schild plot.
[a] Significant difference from unity ($P < 0.05$).

3. Results

3.1. Antagonism between norepinephrine and BMY 7378

Norepinephrine produced concentration-dependent contractions of thoracic and abdominal aorta, mesenteric, renal and iliac arteries with pD_2 values of 7.77 ± 0.04 ($n = 4$), 7.76 ± 0.08 ($n = 4$), 7.02 ± 0.02 ($n = 4$), 7.11 ± 0.04 ($n = 4$) and 8.40 ± 0.02 ($n = 4$), respectively. All the pD_2 values of norepinephrine coincided fundamentally with those reported by Bevan et al. (1986) and Oriowo et al. (1987). The selective α_{1D}-adrenoceptor antagonist, BMY

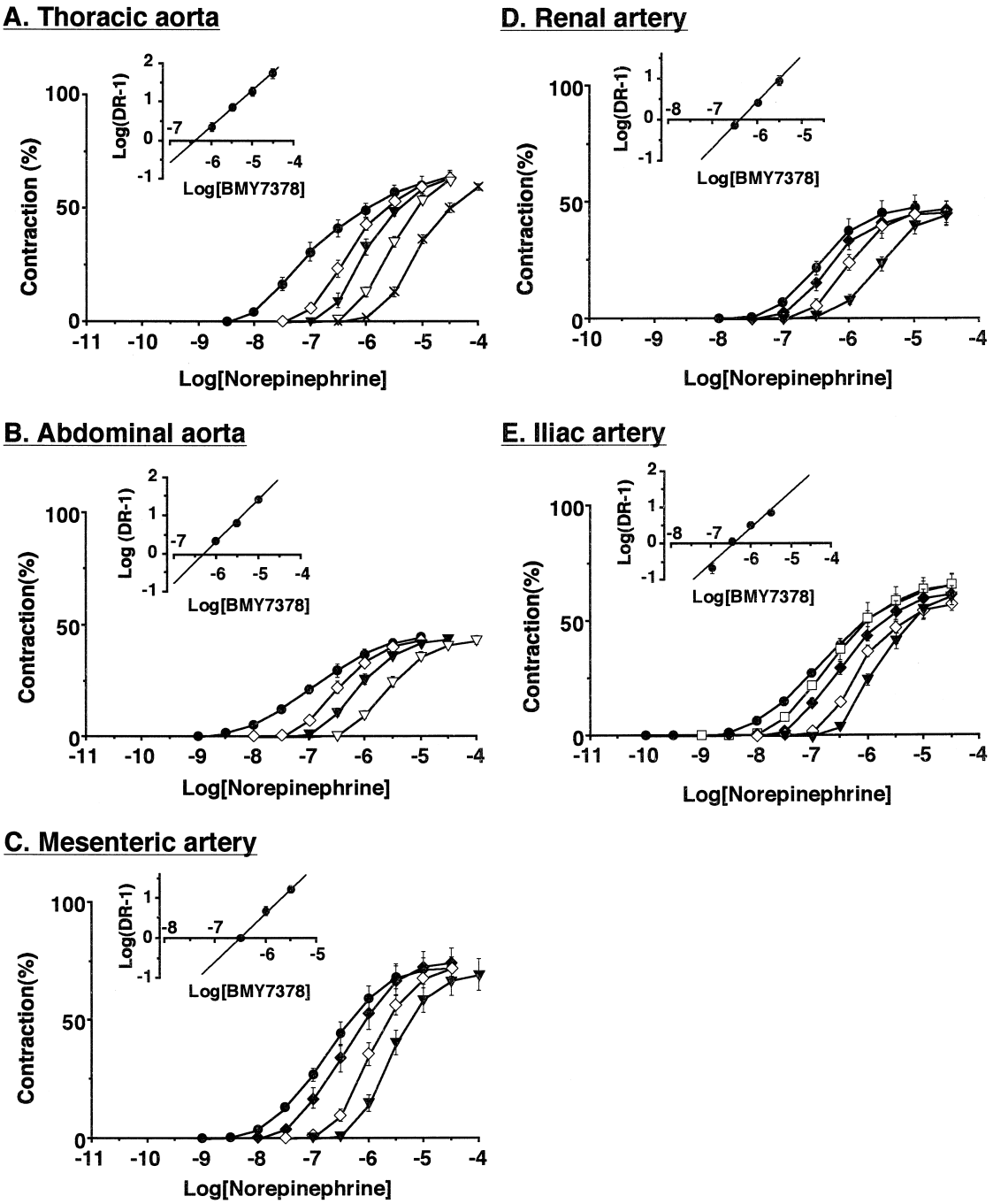

Fig. 3. Effect of BMY 7378 on norepinephrine-induced contraction and Schild plots for antagonism between norepinephrine and this antagonist in Ca^{2+}-free PSS in five arteries of rabbit. (A) Thoracic aorta; (B) abdominal aorta; (C) mesenteric artery; (D) renal artery; (E) iliac artery. Ordinate: Contraction (%) which is expressed as a percentage of the contractile response to norepinephrine (3×10^{-6} M) in normal PSS. Abscissa: logarithm of norepinephrine concentration (M). ●, norepinephrine alone; □, 10^{-7} M BMY 7378; ♦, 3×10^{-7} M BMY 7378; ◇, 10^{-6} M BMY 7378; ▼, 3×10^{-6} M BMY 7378; ▽, 10^{-5} M BMY 7378. Inset, Schild plots. Ordinate: logarithm of equieffective concentration ratio (DR) of norepinephrine minus 1. Abscissa: logarithm of molar concentration of BMY 7378. Each value is presented as the mean ± S.E. (bar).

7378, shifted the concentration–response curves for norepinephrine to the right in five arteries (Fig. 1). In thoracic and abdominal aorta, the Schild regression obtained from the results of antagonism between BMY 7378 and norepinephrine yielded a straight line with a slope of unity (1.05 ± 0.02 and 0.97 ± 0.03), suggesting simple competitive antagonism, and the pA_2 values for BMY 7378 against norepinephrine were 6.54 ± 0.02 and 6.73 ± 0.03, respectively. However, in mesenteric, renal and iliac arteries, Schild plots of the results obtained from the inhibition by BMY 7378 for norepinephrine yielded a slope significantly different from unity (0.57 ± 0.05, 0.61 ± 0.10 and 0.59 ± 0.02), suggesting that norepinephrine acted through at least two receptor populations (Table 1).

In chloroethylclonidine-treated thoracic and abdominal aorta, mesenteric, renal and iliac arteries, norepinephrine induced concentration-dependent contractions with pD_2 values of 6.59 ± 0.03 ($n = 4$), 6.26 ± 0.09 ($n = 4$), 6.26 ± 0.11 ($n = 4$), 7.04 ± 0.18 ($n = 4$) and 6.64 ± 0.17 ($n = 4$), respectively. The maximum response to norepinephrine was not reduced significantly (Fig. 2). BMY 7378 shifted the concentration–response curves for norepinephrine to the right in five arteries. As shown in Fig. 2, with thoracic and abdominal aorta, the Schild regression obtained from the results of antagonism between BMY 7378 and norepinephrine yielded a straight line with a slope of unity, suggesting simple competitive antagonism, and the pA_2 values for BMY 7378 against norepinephrine were 6.49 ± 0.14 ($n = 4$) and 6.61 ± 0.11 ($n = 4$), respectively. However, in mesenteric, renal and iliac arteries, Schild plots of the results obtained from the inhibition by BMY 7378 for norepinephrine yielded a slope significantly different from unity, suggesting that norepinephrine acted through at least two receptor populations. The pA_2 values for BMY 7378 against norepinephrine, estimated from Schild plot analysis and slope of Schild plots, are summarized in Table 2.

In Ca^{2+}-free PSS, strips of arteries respond to norepinephrine with a concentration-dependent contraction. The pD_2 values obtained with thoracic and abdominal aorta, mesenteric, renal and iliac arteries were 6.71 ± 0.16 ($n = 4$), 6.80 ± 0.12 ($n = 4$), 6.31 ± 0.08 ($n = 4$), 6.44 ± 0.02 ($n = 4$) and 6.63 ± 0.07($n = 4$), respectively. The maximum response to norepinephrine was reduced significantly by 25 to 50%. As shown in Fig. 3, the concentration–response curves for norepinephrine were shifted to the right in parallel by BMY 7378. Schild plots yielded a straight line with a slope of unity with five arteries. The pA_2 values of BMY 7378 against norepinephrine estimated by Schild plot analysis are summarized in Table 3.

Table 3
pA_2 Values for BMY 7378 against norepinephrine and slope of Schild plot for antagonism between norepinephrine and BMY 7378 in Ca^{2+}-free PSS in rabbit arteries. Each value is presented as a mean ± S.E. of four separate experiments

Arteries	n	pA_2 Value	Slope
Thoracic aorta	4	6.41 ± 0.09	1.01 ± 0.03
Abdominal aorta	4	6.28 ± 0.07	1.06 ± 0.04
Mesenteric artery	4	6.55 ± 0.06	1.09 ± 0.13
Renal artery	4	6.24 ± 0.10	0.92 ± 0.06
Iliac artery	4	6.64 ± 0.13	0.97 ± 0.08

3.2. Inhibition of [^{3}H]prazosin binding by BMY 7378

BMY 7378 inhibited the binding of [^{3}H]prazosin to five arteries (thoracic and abdominal aorta, mesenteric, renal and iliac arteries) (Fig. 4). With thoracic and abdominal aorta, the pseudo-Hill coefficients for BMY 7378 obtained from Hill plots were 1.03 ± 0.03 and 0.95 ± 0.06, respectively, suggesting that this agent interacts with one site for [^{3}H]prazosin. In mesenteric, renal and iliac arteries, nonlinear regression analysis of the inhibition curves for BMY 7378 in membrane preparations best fitted a two-site model; the $pK_{i\,High}$ values of BMY 7378 for high-affinity binding were 8.66 ± 0.28, 8.71 ± 0.33 and 8.60 ± 0.24, respectively, and the $pK_{i\,Low}$ values of BMY 7378 for low-affinity binding were 6.34 ± 0.14, 6.45 ± 0.03 and 6.56 ± 0.13, respectively. The pK_i values of BMY 7378 and slopes of pseudo-Hill plots are summarized in Table 4.

4. Discussion

Pharmacological studies of the α_{1D}-adrenoceptor subtype had not yet yielded consistent results, although a number of studies have previously concluded that several different α_1-adrenoceptor subtypes contribute to the contractile response of rabbit blood vessels (Tsujimoto et al., 1989; Muramatsu et al., 1990; Suzuki et al., 1990; Satoh et al., 1992a; Leonardi et al., 1997; Suzuki et al., 1997; Testa et al., 1997). One of the sources of confusion about α_{1D}- and α_{1d}-adrenoceptor subtypes may be the comparison of data obtained from animals of different species and strains and from different organs. We studied α_{1D}-adrenoceptor subtypes in the same species and system, the rabbit vascular system, based on data reported for rat thoracic aorta. In the present study with arteries we found evidence for a co-existing α_{1D}-adrenoceptor subtype, which was held not to exist in rabbit blood vessels, and we studied the distribution of this subtype and characterized the receptor-mediated contraction. As shown in Fig. 1D and E, with renal and iliac artery, concentration–response curves for norepinephrine were potently antagonized by low concentrations of BMY 7378. Since the slopes (0.61 ± 0.10 and 0.59 ± 0.02, respectively) of the Schild plots were significantly different from unity, the suggestion arises of the existence of a BMY 7378-sensitive (non-α_{1A}, non-α_{1B}) α_{1D}-adrenoceptor subtype and also of the possibility of a contractile response through α_{1D}-adrenoceptors for norepinephrine-induced contraction of smooth muscle. On the other hand, with thoracic and abdominal aorta, Schild plots of the

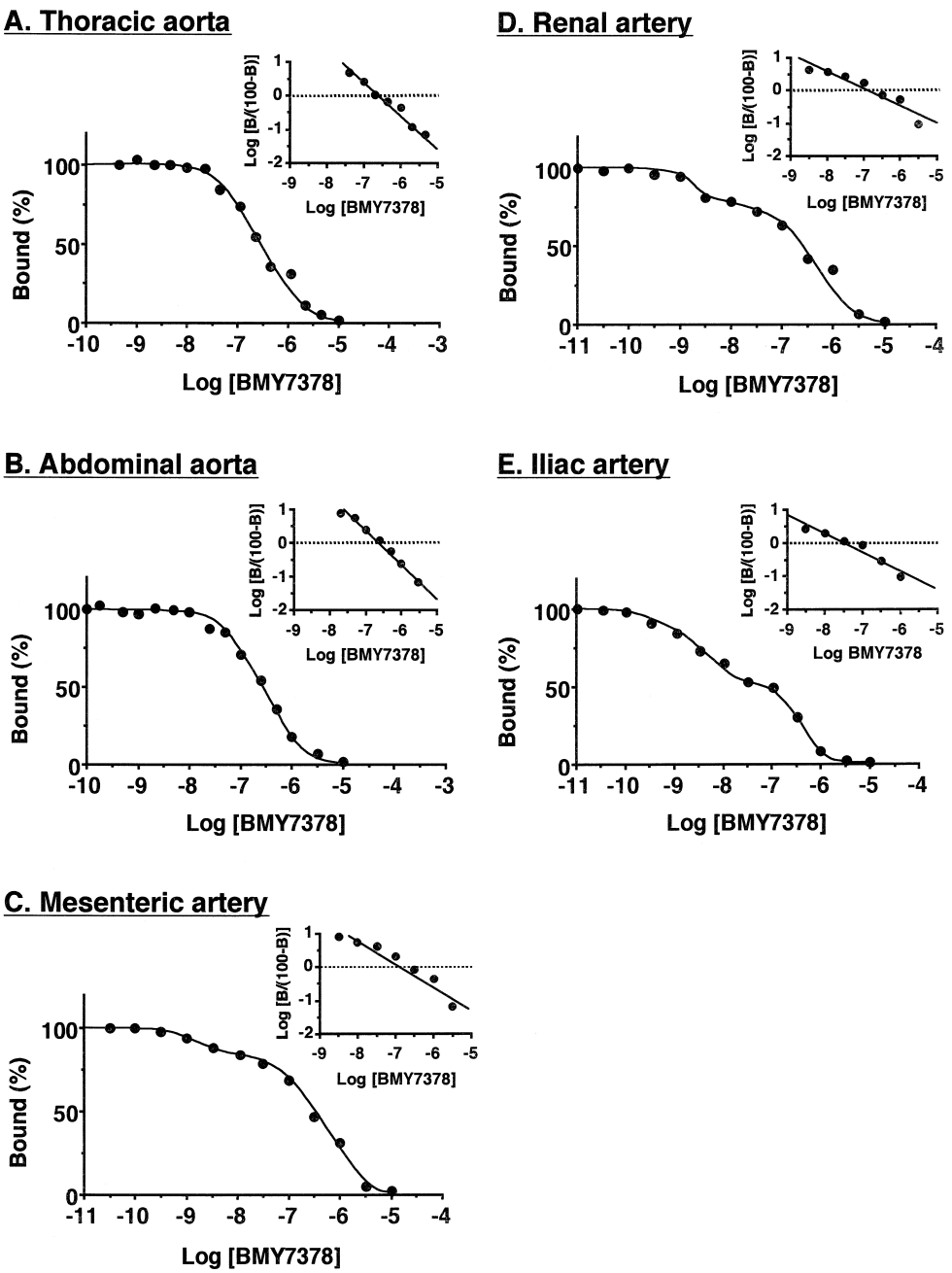

Fig. 4. Inhibition of specific binding of [^{3}H]prazosin to membrane preparations from five arteries of rabbit by BMY 7378. Inset, pseudo Hill plots of the same data. (A) Thoracic aorta; (B) abdominal aorta; (C) mesenteric artery; (D) renal artery; (E) iliac artery. The competition curve generated was best fitted by a two-site model with a pseudo Hill coefficient. The data shown represent three similar experiments.

results obtained from the inhibition by BMY 7378 yielded straight lines with a slope of unity (1.05 ± 0.02 and 0.97 ± 0.03), and the pA_2 values for BMY 7378 were 6.54 ± 0.02 and 6.73 ± 0.03, respectively. These findings differ from those reported for the α_{1D}-adrenoceptor by Kenny et al. (1995), Testa et al. (1995) and Eltze (1997), and are similar to those for α_{1A} or α_{1B}. These findings suggest that the α_1-adrenoceptor-mediating contraction in thoracic and abdominal aorta is not related to the α_{1D}-adrenoceptor subtype. As reported previously (Satoh et al., 1992a,b), it is the α_{1A}- and α_{1B}-adrenoceptor subtypes that contribute to the norepinephrine-induced contraction. Goetz et al. (1995) and Kenny et al. (1995) reported that BMY 7378 was a selective α_{1D}-adrenoceptor antagonist and possessed an approximately greater than 100-fold higher affinity for the α_{1D}-adrenoceptor than for the α_{1A}- and α_{1B}-adrenoceptors in cells expressing cloned human or non-human and rat thoracic aorta native α_1-adrenoceptors. As shown in Fig. 1E, in iliac artery concentration–response curves for norepinephrine were potently antagonized by BMY 7378,

Table 4
BMY 7378 competition for [^{3}H]prazosin binding sites to membrane preparations from rabbit arteries. Inhibition studies were conducted as described in Fig. 4. $pK_i = -\log K_i$ calculated from IC_{50} values according to Cheng and Prusoff (1973). n_H = pseudo Hill coefficient. N.D. = not detectable. Each value is presented as a mean ± S.E. of three separate experiments

Arteries	n	n_H	$pK_{i\,High}$ (α_{1D})	$pK_{i\,Low}$ ($\alpha_{1A,1B\ or\ 1L}$)	% High affinity sites
Thoracic aorta	3	1.03 ± 0.03	N.D.	6.44 ± 0.08	N.D.
Abdominal aorta	3	0.95 ± 0.06	N.D.	6.59 ± 0.02	N.D.
Mesenteric artery	3	0.61 ± 0.09[a]	8.66 ± 0.28	6.34 ± 0.14	18.8 ± 6.05
Renal artery	3	0.50 ± 0.08[a]	8.71 ± 0.33	6.45 ± 0.03	30.7 ± 3.18
Iliac artery	3	0.66 ± 0.02[a]	8.60 ± 0.24	6.56 ± 0.13	45.3 ± 5.93

[a] Significant difference from the corresponding value ($P < 0.05$).

because the intercept of the Schild plot was approximately 8.41 which is similar to the value (pK_B 8.3) reported by Kenny et al. (1995). In renal artery, BMY 7378 antagonizes the high-affinity portion (below 30%) of concentration–response curves for norepinephrine more potently than the low-affinity portion (above 50%) of the concentration–response curves. Schild regression analysis at 20% contraction also yielded a slope significantly different from unity (0.62), and the intercept was approximately 8.50. Chloroethylclonidine-treated preparations yielded similar results. As shown in Fig. 2E, in iliac artery the concentration–response curves for norepinephrine were antagonized by BMY 7378. Schild plots of the results yielded a slope significantly different from unity, and the intercept of the regression line was approximately 8.42, suggesting that norepinephrine acted through at least two receptor populations and that one of these was an α_{1D}-adrenoceptor subtype. In chloroethylclonidine-treated renal artery, BMY 7378 antagonizes the high-affinity portion (below 50%) of the concentration–response curve for norepinephrine more potently than the low-affinity portion (above 50%) of the concentration–response curves (Fig. 2D). Schild regression analysis at 20% contraction also yielded a slope significantly different from unity (0.69), and the intercept was approximately 8.61. As shown in Fig. 2A, B and Table 2, in chloroethylclonidine-treated thoracic and abdominal aorta, the Schild regression obtained from the results of the antagonism between BMY 7378 and norepinephrine yielded a straight line with a slope of unity (0.97 ± 0.02 and 1.03 ± 0.03), suggesting simple competitive antagonism. The pA_2 values for BMY 7378 against norepinephrine were 6.49 ± 0.14 and 6.61 ± 0.11, respectively. Further, in chloroethylclonidine-treated and untreated mesenteric artery, the concentration–response curves for norepinephrine were also antagonized by a high concentration of BMY 7378. However, Schild plots of the results yielded a slope (untreated 0.57 ± 0.05 and chloroethylclonidine-treated 0.47 ± 0.07) significantly different from unity and the intercept of the regression line was approximately 6.5, which is lower than the value for α_{1D}-adrenoceptor subtypes, suggesting that norepinephrine acted through at least two receptor populations that exclude the α_{1D}-adrenoceptor subtype. This value is similar to the pA_2 value (6.18) reported by Van der Graaf et al. (1997). These authors reported that the antagonistic effects of prazosin and HV 723 are of a low potency and that the functional receptors in mesenteric artery belonged to an α_{1L}-adrenoceptor subtype that differs from the α_{1D}-adrenoceptor subtype. In mechanical experiments with mesenteric artery, we also obtained a pA_2 value (8.54 ± 0.03, $n = 4$) for prazosin that was low potency. In the present functional study using BMY 7378, with iliac and renal arteries, the α_1-adrenoceptor-mediated contractions had pharmacological characteristics of the α_{1D}-adrenoceptor subtype, but thoracic and abdominal aorta and mesenteric arteries did not have a functional α_{1D}-adrenoceptor subtype.

In addition to our functional study, we found that BMY 7378 also inhibited [^{3}H]prazosin binding to membranes of thoracic and abdominal aorta, mesenteric, renal and iliac artery (Fig. 4). In thoracic and abdominal aorta, BMY 7378 had only low-affinity sites ($pK_i \sim 6.5$), which were very close to their pA_2 values (6.54 ± 0.02 and 6.73 ± 0.03). BMY 7378 could inhibit with low potency [^{3}H]prazosin binding to membrane preparations from prostate and salivary gland, which primarily contain α_{1A}-adrenoceptors (Michel et al., 1989; Yazawa and Honda, 1993) as well as to liver membranes, which contain α_{1B}-adrenoceptors (Han et al., 1987; Faure et al., 1994). BMY 7378 has a low affinity for cloned α_{1a}- (pK_i 6.2 ± 0.10) and α_{1b}-adrenoceptors (pK_i 6.7 ± 0.11) (Kenny et al., 1995). The findings are also supported by the observation of two α_1-adrenoceptor subtypes, α_{1A} and α_{1B}, in smooth muscle of rabbit aorta (Suzuki et al., 1990; Satoh et al., 1992a,b). On the other hand, in mesenteric, renal and iliac artery, BMY 7378 has two binding sites; one is a high-affinity site ($pK_{i\,High} \sim 8.6$), which is possibly an α_{1D}-adrenoceptor subtype. Goetz et al. (1995) and Kenny et al. (1995) demonstrated that BMY 7378 displayed high affinity (pK_i 8.2) for cloned human α_{1D}-adrenoceptors. In addition these $pK_{i\,High}$ values are very close to pK_B (8.3 ± 0.1) or pA_2 (9.0 ± 0.13) values against muscle contraction with norepinephrine on rat aorta (Kenny et al., 1995; Deng et al., 1996). In our functional study of iliac artery the intercept of the Schild plot was approximately 8.41 which is high potency for BMY 7378 (Fig. 1E). The other is a low-affinity site ($pK_{i\,Low} \sim 6.5$), which is possibly an α_{1A}-, α_{1B}- or α_{1L}-adrenoceptor subtype. These $pK_{i\,Low}$ values are the same as those obtained from thoracic

and abdominal aorta (Table 4), and also are very close to their pA_2 values obtained from the contraction experiment. Based on the [^{3}H]prazosin binding study, it is suggested that α_{1D}-adrenoceptors co-exist in mesenteric, renal and iliac arteries in addition to α_{1A}- and α_{1B}-adrenoceptor subtypes.

In the present study, having observed the existence of an α_{1D}-adrenoceptor in blood vessels of rabbit and having noticed regional differences in α_1-adrenoceptors, we can go on to clarify the receptor mechanisms for contraction of smooth muscle. As shown in Fig. 3, in the five rabbit arteries studied, the potency (pD_2 value) and the maximum response decreased in Ca^{2+}-free PSS, suggesting that norepinephrine-induced contraction is partly the result of a Ca^{2+} influx originating from extracellular Ca^{2+}. Concentration–response curves for norepinephrine in all arteries in Ca^{2+}-free PSS were shifted in parallel by BMY 7378. Schild plots of these results yielded straight lines with a slope of unity, suggesting simple competitive antagonism. The pA_2 values for the antagonist against norepinephrine were 6.24 to 6.64 which is significantly smaller than that ($\sim$ 8.3) reported for the α_{1D}-adrenoceptor subtype (Kenny et al., 1995; Deng et al., 1996; Villalobos-Molina and Ibarra, 1996; Eltze, 1997). As with normal PSS, as mentioned above (Fig. 1), Schild plots of the renal and iliac artery results obtained from the inhibition by BMY 7378 for norepinephrine yielded a slope significantly different from unity. From the comparison of mesenteric, renal and iliac artery, in which the α_{1D}-adrenoceptor subtype exists, renal and iliac artery are the preparations with the highest portion of high-affinity sites (α_{1D}) observed (30.7 and 45.3%, respectively), which is consistent with the observation that these vessels are also highly sensitive (decrease in maximum contraction $>$ 30%) to the removal of extracellular Ca^{2+}, whereas this seems to be less important for mesenteric artery (18.8% high affinity sites), in which removal of extracellular Ca^{2+} results in depression of approximately 25%. There seems to be a correlation between the amount of high-affinity sites and the magnitude of the pA_2 values of BMY7378 determined in untreated and chloroethylclonidine-treated tissues: iliac artery = renal artery $>$ mesenteric artery. It will be clear from these observations that the contractile pathway involving the α_{1D}-adrenoceptor subtype in smooth muscle of renal and iliac arteries is specifically blocked by the removal of Ca^{2+} from PSS. That is to say, in Ca^{2+}-free PSS the norepinephrine-induced contraction which is produced by α_{1D}-adrenoceptor subtypes is mainly due to influx of Ca^{2+} from the extracellular Ca^{2+}. The norepinephrine-induced contraction in Ca^{2+}-free PSS is due to the Ca^{2+} released from intracellular Ca^{2+} stores, and is produced through α_{1B}-adrenoceptor subtypes, since the pA_2 values are similar to those for α_{1A}- and α_{1B}-adrenoceptor subtypes and since it is reported that the α_{1A}-adrenoceptor mediation of contraction is through a Ca^{2+} influx through L-type Ca^{2+} channels (Suzuki et al., 1990; Satoh et al., 1998). Furthermore, the amplitude of the α_{1D}-adrenoceptor-mediated contraction in rat thoracic aorta in Ca^{2+}-free PSS was 10 to 20% of that in normal PSS (data not shown). It therefore seems reasonable to suppose, based on previous observations, that α_{1A}- and α_{1D}-adrenoceptor subtypes induce a Ca^{2+} influx accelerated by the opening of Ca^{2+} channels, thereby producing an extracellular Ca^{2+}-dependent contraction in arteries of rabbit, whereas α_{1B}-adrenoceptor subtypes produce an intracellular Ca^{2+}-dependent contraction which is stimulated by intracellular Ca^{2+} mobilization from Ca^{2+} stores.

In summary, the data of this study suggest that α_{1D}-adrenoceptor subtypes co-exist in mesenteric, renal and iliac artery of rabbit, and that renal and iliac artery, in response to norepinephrine, contract via the activation of α_{1D}-adrenoceptor subtypes added to α_{1A}- and α_{1B}-adrenoceptor subtypes, whereas thoracic and abdominal aorta contract via the activation of α_{1A}- and α_{1B}-adrenoceptor subtypes. α_{1D}-Adrenoceptor subtypes have pharmacological characteristics which accelerate the influx of extracellular Ca^{2+}. α_{1D}-Adrenoceptors play an important role in norepinephrine-induced muscle contraction in renal and iliac artery, but have virtually no role in mesenteric artery, even though α_{1D}-adrenoceptor subtypes do exist in this tissue. Also α_{1D}-adrenoceptors are not detectable from norepinephrine-induced contractions and [^{3}H]prazosin binding experiments with thoracic and abdominal aorta. In addition, it is also suggested that an α_1-adrenoceptor subtype, which is pharmacologically distinguishable from the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtype, may co-exist in mesenteric artery of rabbit.

Acknowledgements

This study was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (Nos. 09772003 and 11672191).

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