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Regional differences in α_1-adrenoceptor subtypes and mechanisms in rabbit arteries

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Abstract

Contractility mediated through α_1-adrenoceptor subtypes and the maximum binding site (B_{max} value) and the dissociation constant (K_d value) for [^{125}I]HEAT ([^{125}I]iodo-2-(β-(4-hydroxyphenyl)ethylaminomethyl)tetralone) were determined in the following rabbit arteries: thoracic and abdominal aorta, mesenteric, renal and iliac arteries, and the α_1-adrenoceptor subtypes mediating contractile mechanisms in vascular smooth muscle were studied. The pD_2 values for norepinephrine differed considerably among the arteries in the presence of nicardipine (10^{-5} M), while the pA_2 values for 5-methylurapidil against norepinephrine were identical at low affinity in all the arteries used. In Ca^{2+}-free physiological saline solution (Ca^{2+}-free PSS), the pA_2 values for 5-methylurapidil were also similar except for the renal artery, in which there were no stable contractions. In normal PSS, the concentration–response curves for norepinephrine with chloroethylclonidine-pretreatment were shifted to the right (pD_2 values of 5.58, 5.70, 5.74, 5.98 and 6.38 for thoracic and abdominal aorta, mesenteric, renal and iliac arteries, respectively). In the [^{125}I]HEAT binding study using membrane preparations obtained from chloroethylclonidine-treated strips, the B_{max} values (33.2–105.2 fmol/mg protein) for [^{125}I]HEAT varied considerably among arteries, while the K_d values (0.20–0.26 nM) were identical. The logarithm of B_{max} values is proportional to the pD_2 values for norepinephrine (slope = 0.69, $r = 0.961$). These observations suggest that the regional differences in potency (pD_2 value) of the α_1-adrenoceptor agonist, norepinephrine, are a result of the differences in population and density of α_1-adrenoceptor subtypes in rabbit arteries.

Keywords: α_1-Adrenoceptor; 5-Methylurapidil; [^{125}I]HEAT ([^{125}I]iodo-2-(β-(4-hydroxyphenyl)ethylaminomethyl)tetralone); Regional difference; Artery; Rabbit

1. Introduction

Pharmacological studies have consistently demonstrated the existence of at least two different α_1-adrenoceptor subtypes, α_{1A} and α_{1B}, in various tissues. Currently, α_1-adrenoceptors are pharmacologically divided into three major subtypes, α_{1A}, α_{1B} and α_{1D} subtypes, based on differences in their affinities for selective competitive agonists and an irreversible antagonist. The α_{1A}-subtype has a high affinity for antagonists, WB4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4 benzodioxane), 5-methylurapidil, (+)-niguldipine (Boer et al., 1989; Gross et al., 1989; Tsujimoto et al., 1989), while the α_{1B}-subtype is irreversibly inactivated by an alkylating agent, chloroethylclonidine (Han et al., 1987), and the α_{1D}-subtype has a high affinity for the antagonist, BMY7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)lethyl)-8-azapirol(4,5)decane-7,9-dione 2HCl) (Saussy et al., 1994). Molecular cloning studies have done much to support this classification and the three subtypes (α_{1A}, α_{1B} and α_{1D}) have been identified (Bylund et al., 1994). Recently these three native subtypes α_{1A}, α_{1B} and α_{1D} have been designated as corresponding to cloned subtypes α_{1c}, α_{1b} and $\alpha_{1a/d}$, respectively (Hieble et al., 1995).

Tsujimoto et al. (1989), Takayanagi et al. (1991a) and Satoh et al. (1992a,b) showed that the thoracic aorta and iliac artery of rabbit contain α_{1A}- and α_{1B}-subtypes. Each receptor subtype has a distinct role: α_{1A}-subtypes cause a tonic response predominantly dependent on the influx of extracellular Ca^{2+}, whereas α_{1B}-subtypes cause a phasic

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Table 1
Effects of nicardipine (10^{-5} M) on pD_2 values and maximum responses for norepinephrine in rabbit arteries

Arteries	n	Untreated	Nicardipine-treated	
		pD_2	pD_2	Maximum response (%)
Thoracic aorta	4	7.08 ± 0.04	6.75 ± 0.03[a]	89.5 ± 3.43
Abdominal aorta	7	6.96 ± 0.12	6.63 ± 0.12	87.6 ± 7.96
Mesenteric artery	5	6.53 ± 0.10	6.10 ± 0.18	62.0 ± 2.28[b]
Renal artery	5	6.89 ± 0.19	5.80 ± 0.11[a]	43.5 ± 5.05[b]
Common iliac artery	4	7.49 ± 0.19	6.64 ± 0.25[a]	68.0 ± 2.29[b]

Each value is presented as a mean ± S.E. Maximum response is expressed as a percentage of the contractile response to norepinephrine (3×10^{-6} M) in the absence of nicardipine.
[a,b]Significant difference from the corresponding value ($P < 0.05$).

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). Satoh et al. (1994) and Kokubu et al. (1995) stated that Ca^{2+} sensitization produced by α_{1A}-subtypes is mediated through G-protein and protein kinase C, and plays an important role in the contraction of rabbit thoracic aorta. These reports suggest that muscle contractility in the physiological vascular system is regulated by signal transduction pathways from a heterogeneous population of coexisting α_{1A}- and α_{1B}-adrenoceptors.

To clarify the relationship between the heterogenous population of α_1-adrenoceptor subtypes and the regional differences in potency (pD_2 value) for α_1-adrenoceptor agonists, we studied pharmacologically the contractile characteristics of vascular smooth muscles produced by each subtype of heterogenous α_1-adrenoceptor, and the physiological roles that these receptors play in the contractile responses of rabbit arteries. We also conducted binding experiments using [^{125}I]HEAT ([^{125}I]iodo-2-(β-(4-hydroxyphenyl) ethylaminomethyl)tetralone) in vessels from five different regions (thoracic and abdominal aorta, mesenteric, renal and iliac arteries) of rabbit.

2. Materials and 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. Each artery was quickly removed and dissected free of excess fat and connective tissue in oxygenated physiological saline solution (PSS) of the following composition (in mmol): 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 ethyleneglycol 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 used were thoracic and abdominal aorta and mesenteric, renal and iliac arteries. 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. After determination of the control concentration–response curves for agonists, 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

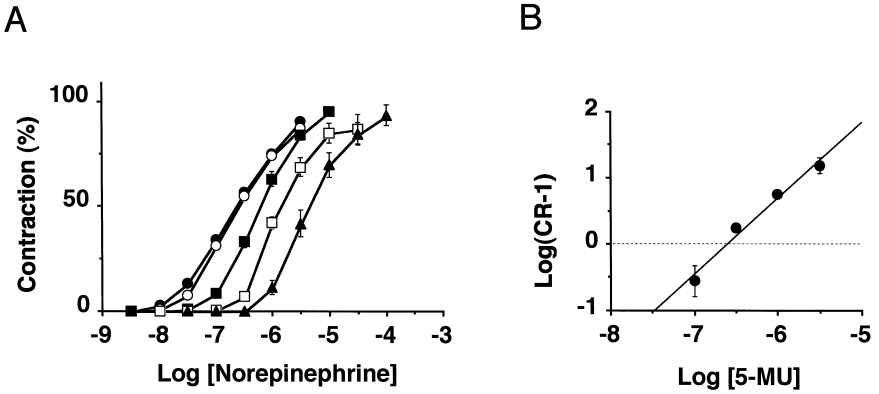

Fig. 1. Antagonistic effects of 5-methylurapidil (A), and Schild plot (B) for antagonism between norepinephrine and this antagonist in the presence of nicardipine (10^{-5} M) in the rabbit thoracic aorta. In A, Ordinate: contraction (%) which is expressed as a percentage of the contractile response to norepinephrine (3×10^{-6} M) in the absence of nicardipine. Abscissa: logarithm of norepinephrine concentration (M). ●, agonist alone; ○, 10^{-7} M 5-methylurapidil; ■, 3×10^{-7} M 5-methylurapidil; □, 10^{-6} M 5-methylurapidil; ▲, 3×10^{-6} M 5-methylurapidil. In B, Ordinate: logarithm of equieffective concentration ratio (CR) of norepinephrine minus 1. Abscissa: logarithm of molar concentration of 5-methylurapidil (5-MU). Each value is presented as the mean ± S.E. (bar) of four experiments.

Table 2
pA_2 values for 5-methylurapidil against norepinephrine and slope of Schild plot for antagonism between norepinephrine and 5-methylurapidil in the presence of nicardipine (10^{-5} M) in rabbit arteries

Arteries	*n*	pA_2	Slope
Thoracic aorta	4	6.77 ± 0.14	1.01 ± 0.06
Abdominal aorta	4	6.73 ± 0.15	0.89 ± 0.11
Mesenteric artery	4	7.06 ± 0.12	1.03 ± 0.23
Renal artery	8	6.92 ± 0.10	0.98 ± 0.13
Common iliac artery	4	7.05 ± 0.16	0.95 ± 0.03

Each value is presented as a mean ± S.E.

the same time. The concentration–response curves for agonists were made after sufficient repetitions had been done to confirm that constant curves had been established.

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. This was repeated until two successive contractions of approximately equal size had been obtained. The 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) concentration of the antagonist and the same preparation. 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. To exclude α_{1B}-adrenoceptor subtypes, all tissues were treated with 10 μM chloroethylclonidine for a total of 60 min; this antagonist was renewed every 10 min to allow for decomposition of the drug in the solution. 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) and used for the binding assay.

2.4. [^{125}I]HEAT binding

Membranes prepared from each rabbit artery were incubated with 0.025–0.4 nM [^{125}I]HEAT in an assay buffer for 30 min at 4°C. 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

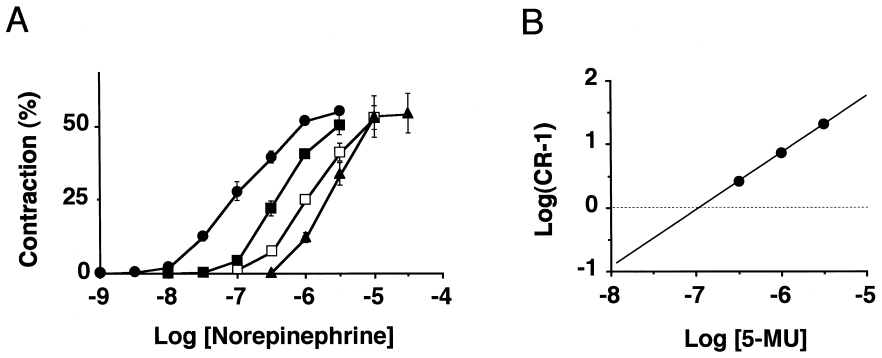

Fig. 2. Antagonistic effects of 5-methylurapidil (A), and Schild plot (B) for antagonism between norepinephrine and this antagonist in Ca^{2+}-free PSS in the rabbit thoracic aorta. (A) Ordinate: contraction (%) which is expressed as a percentage of the contractile response to norepinephrine (3×10^{-6} M) in normal PSS. Abscissa: logarithm of norepinephrine concentration (M). ●, agonist alone; ■, 3×10^{-7} M 5-methylurapidil; □, 10^{-6} M 5-methylurapidil; ▲, 3×10^{-6} M 5-methylurapidil. (B) Ordinate: logarithm of equieffective concentration ratio (CR) of norepinephrine minus 1. Abscissa: logarithm of molar concentration of 5-methylurapidil (5-MU). Each value is presented as the mean ± S.E. (bar) of four experiments.

Table 3
pA_2 values for 5-methylurapidil against norepinephrine and slope of Schild plot for antagonism between norepinephrine and 5-methylurapidil in Ca^{2+}-free PSS in rabbit arteries

Arteries	*n*	pA_2	Slope
Thoracic aorta	4	6.93 ± 0.15	0.92 ± 0.05
Abdominal aorta	4	7.10 ± 0.26	0.98 ± 0.01
Mesenteric artery	4	6.83 ± 0.17	1.10 ± 0.10
Renal artery		–	–
Common iliac artery	4	7.12 ± 0.27	1.10 ± 0.25

Each value is presented as a mean ± S.E.

overnight in scintillation fluid and the radioactivity was determined in a liquid scintillation counter. Specific [^{125}I]HEAT binding was determined experimentally from the difference between counts in the absence and presence of 10 μM phentolamine. All assays were done in duplicate. The apparent dissociation constant (K_d) and B_{max} for [^{125}I]HEAT were estimated by Scatchard analysis of the saturation data over a concentration range of 0.025 to 0.4 nM (Scatchard, 1949). The Hill coefficient for saturation data for [^{125}I]HEAT was obtained from the Hill plot. The data obtained 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 with an *F*-test, 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-methylurapidil and chloroethylclonidine (Research Biochemicals, Natick, MA); desmethylimipramine hydrochloride, (±)-normetanephrine hydrochloride, (±)-propranolol hydrochloride, and yohimbine hydrochloride (Sigma), pentobarbital sodium (Abbott Lab., North Chicago, IL, USA) and [^{125}I]HEAT (specific activity 2200 Ci/mmol, NEN, Boston, USA). Other chemicals used were of analytical grade.

3. Results

3.1. Antagonism between norepinephrine and 5-methylurapidil

All the helical strips of arteries responded to norepinephrine with concentration-dependent contraction. All the pD_2 values of norepinephrine coincided fundamentally with those reported by Bevan et al. (1986) and Oriowo et al. (1987). Concentration–response curves for norepinephrine were shifted to the right by the Ca^{2+} channel antagonist, nicardipine. In mesenteric, renal and common iliac arteries, the maximum response to norepinephrine was reduced significantly by 30 to 55%, but in thoracic and abdominal aorta only approximately 10% of maximum response was lost (Table 1). As shown in Fig. 1, in the presence of nicardipine, the concentration–response curves for norepinephrine were shifted in parallel by an α_1-adrenoceptor antagonist, 5-methylurapidil (10^{-7}, 3×10^{-7}, 10^{-6} and 3×10^{-6} M), with each strip. Schild plots yielded a straight line with a slope of 1. The pA_2 values for 5-methylurapidil against norepinephrine estimated by Schild plot analysis are summarized in Table 2.

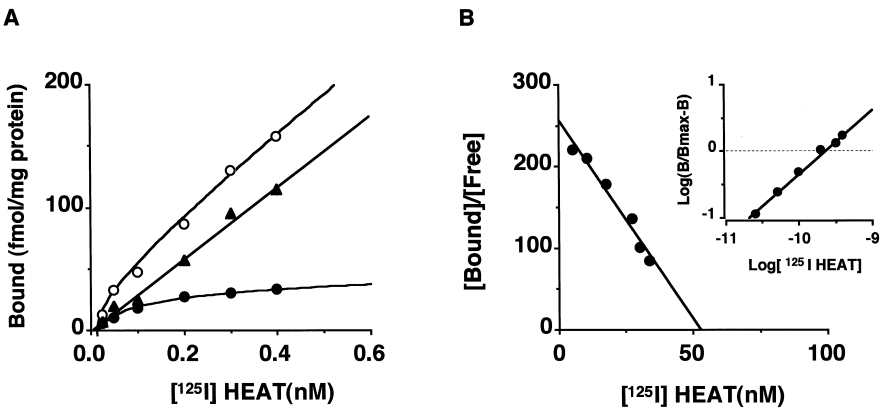

Fig. 3. Saturation binding (A), Scatchard plot (B) and Hill plot (C) of [^{125}I]HEAT to membranes prepared from chloroethylclonidine-treated rabbit thoracic aorta. Increasing concentrations of [^{125}I]HEAT (0.025–0.4 nM) were bound to membranes. Specific binding was defined as that which yielded a one-site model ($K_d = 0.23 \pm 0.05$ nM, $B_{max} = 33.2 \pm 8.76$ fmol/mg protein, Hill coefficient = 0.96 ± 0.05). The data shown are means of triplicate determinations in a representative experiment. (○) Total binding; (▲) non-specific binding; (●) specific binding. Tissues were pretreated with chloroethylclonidine (10^{-4} M) for 60 min.

Table 4
Maximum binding site (B_{max}), dissociation constant (K_d) and slope of Hill plot obtained from specific binding of [^{125}I]HEAT to membranes prepared from chloroethylclonidine-treated rabbit arteries

Arteries	n	B_{max} (fmol/mg protein)	K_d (nM)	Hill coefficient
Thoracic aorta	4	33.2 ± 8.76	0.23 ± 0.05	0.96 ± 0.05
Abdominal aorta	5	33.6 ± 7.86	0.20 ± 0.04	1.04 ± 0.02
Mesenteric artery	4	42.4 ± 12.8	0.20 ± 0.03	0.98 ± 0.03
Renal artery	4	68.5 ± 10.9	0.23 ± 0.24	0.98 ± 0.03
Common iliac artery	4	105.2 ± 5.88	0.26 ± 0.06	0.97 ± 0.02

Each value is presented as a mean ± S.E. Tissues were pretreated with chloroethylclonidine (10^{-5} M) for 60 min.

In Ca^{2+}-free PSS, strips of arteries (thoracic and abdominal aorta, mesenteric and common iliac artery) responded to norepinephrine with a concentration-dependent contraction, but renal artery strips did not yield stable concentration–response curves. As shown in Fig. 2 for thoracic aorta, the concentration–response curves for norepinephrine with these four arteries were shifted to the right in parallel by 5-methylurapidil (3×10^{-7}, 10^{-6} and 3×10^{-6} M). Schild plots yielded a straight line with a slope of 1. The pA_2 values of 5-methylurapidil against norepinephrine estimated by Schild plot analysis are summarized in Table 3. In normal PSS, the concentration–response curves for norepinephrine with the chloroethylclonidine-pretreatment were shifted to the right. The pD_2 values obtained with thoracic and abdominal aorta, mesenteric, renal and iliac arteries were 5.58 ± 0.03, 5.70 ± 0.06, 5.74 ± 0.04, 5.98 ± 0.11 and 6.38 ± 0.06 ($n = 4$), respectively.

3.2. Saturation binding of [^{125}I]HEAT to membrane preparations from chloroethylclonidine-treated arteries

Binding of [^{125}I]HEAT was studied using membrane preparations obtained from chloroethylclonidine-treated strips (see Section 2). Specific binding of [^{125}I]HEAT (0.025–0.4 nM) in the membrane preparations from chloroethylclonidine-treated thoracic aorta was saturable, with a plateau between 0.3–0.4 nM [^{125}I]HEAT (Fig. 3A). However, non-specific binding increased linearly with the [^{125}I]HEAT concentration. The Scatchard plot (Fig. 3B) was linear and the Hill coefficient ($n_H = 0.96 \pm 0.05$) was not significantly different from unity, suggesting a single population of binding sites with a K_d value of 0.23 ± 0.05 nM and B_{max} of 33.2 ± 8.76 fmol/mg protein. The results for saturation binding of [^{125}I]HEAT in arteries (thoracic, abdominal, mesenteric, renal and common iliac) are summarized in Table 4.

4. Discussion

In the present study, we have shown that, in five arteries of rabbits: (1) the contraction mediated through α_{1A}-adrenoceptor subtypes is mainly due to Ca^{2+} influx induced by the activation of L-type voltage-dependent Ca^{2+} channels; (2) the regional differences in tissue sensitivity to norepinephrine are due to the differences in the population and density of α_1-adrenoceptor subtypes. These points will be discussed separately, beginning with the contractile mechanisms involving α_{1A}-adrenoceptor subtypes.

4.1. α_{1A}-adrenoceptor subtypes mediating contractile mechanisms

In the five rabbit studied arteries, the potency (pD_2 value) decreased in the presence of the Ca^{2+} channel antagonist, nicardipine (10^{-5} M), suggesting that norepinephrine-induced contraction is partly the result of Ca^{2+} influx mediated through L-type Ca^{2+} channels. Concentration–response curves for norepinephrine in all arteries in the presence of nicardipine were shifted in parallel by 5-methylurapidil. Schild plots of these results yield straight lines with a slope of unity, suggesting a simple competitive antagonism. The pA_2 values for the antagonist against norepinephrine were 6.77 to 7.06 which is significantly smaller than that (~ 9) of the α_{1A}-subtype reported (Aboud et al., 1993; Burt et al., 1995). On the other hand, with normal PSS (in the absence of a Ca^{2+} channel antagonist) norepinephrine-induced contractions were produced through both α_{1A}- and α_{1B}-adrenoceptor subtypes in thoracic and common iliac arteries (Takayanagi et al., 1991a; Satoh et al., 1992a,b). With all five arteries, Schild plots of the results obtained from the inhibition by 5-methylurapidil for norepinephrine in normal PSS yielded a slope significantly different from unity (data not shown). It will be clear from these observations that the contractile pathway involving the α_{1A}-adrenoceptor subtype in smooth muscle is specifically blocked by the Ca^{2+} channel antagonist. That is to say, in the presence of nicardipine, the norepinephrine-induced contraction which is produced by α_{1B}-adrenoceptor subtypes is mainly due to Ca^{2+} that is released from the intracellular Ca^{2+} store. Furthermore, in Ca^{2+}-free PSS, 5-methylurapidil also shifted the concentration–response curve for norepinephrine to the right in a parallel manner, and Schild plots of the results yielded straight lines with a slope of unity (Fig. 2 and Table 3), suggesting simple competitive antagonism. The pA_2 val-

ues for 5-methylurapidil in four of the arteries were 6.83 to 7.12, which is significantly different from the value for α_{1A}-subtypes. These observations suggest that norepinephrine-induced contraction in Ca^{2+}-free PSS is produced through α_{1B}-adrenoceptor subtypes, and also that this contraction is due to the Ca^{2+} released from intracellular Ca^{2+} stores. Suzuki et al. (1990) reported that α_{1A}-subtypes cause a tonic response predominantly dependent on the influx of extracellular Ca^{2+}, whereas α_{1B}-subtypes induce intracellular Ca^{2+} mobilization stimulated by phosphatidylinositol hydrolysis and cause a phasic response mainly independent of extracellular Ca^{2+}. It therefore seems reasonable to suppose, based on previous observations that α_{1B}-subtypes produce an intracellular-Ca^{2+}-dependent contraction which is stimulated by intracellular Ca^{2+} mobilization from Ca^{2+} stores, whereas α_{1A}-subtypes induce a Ca^{2+} influx accelerated by the opening of L-type Ca^{2+} channels, thereby producing an extracellular-Ca^{2+}-dependent contraction in arteries of rabbit.

4.2. Regional differences in contractile sensitivity and receptor density

Having observed the co-existence of α_1-adrenoceptor subtypes and having noticed intracellular signal transductions mediated through each subtype, the question arises on site differences in the potency of the α_1-adrenoceptor agonist, norepinephrine, in arteries. As shown in Table 1 in the present studies, the regional differences in potency (pD_2 value) were observed in arteries without chloroethylclonidine treatment. The regional differences in potency persisted in the chloroethylclonidine-treated arteries, i.e., with α_{1B}-adrenoceptor inactivated. The potency order of pD_2 values was: common iliac > renal > mesenteric ≥ abdominal > thoracic artery. As shown in Table 4, this potency order is in good agreement with the B_{max} order observed in the [^{125}I]HEAT binding experiment and chloroethylclonidine-treated preparations. Also the potency (pD_2) of norepinephrine correlates well with the logarithm of B_{max} observed from [^{125}I]HEAT binding (Fig. 4). These observations suggest that after chloroethylclonidine treatment the regional differences in potency of norepinephrine are due to the density of α_{1A}-adrenoceptor subtypes. For chloroethylclonidine-untreated strips, Griendling et al. (1984) reported similarly that the differences in contractile response of canine arteries were due to receptor density. Takayanagi et al. (1987) also reported that, in canine veins, the regional differences in the potency (pD_2 value) of norepinephrine is not due to the affinity of the α_1-adrenoceptors for the agonist, norepinephrine, but to receptor densities. Takayanagi et al. (1991b) suggested that the regional differences in pD_2 values for norepinephrine in rabbit arteries is due to variations in the affinities to the α_1-adrenoceptors as well as to receptor densities. This suggestion was based on results of a study on contraction after partial inactivation by phenoxybenzamine and of a

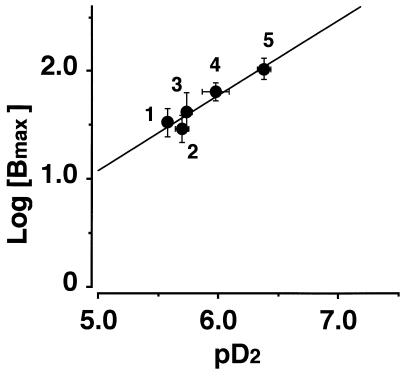

Fig. 4. Relationship between pD_2 value of norepinephrine and the logarithm of the maximum number of binding sites (B_{max}) for [^{125}I]HEAT, obtained from five different arteries of rabbit, all chloroethylclonidine-treated. (1) Thoracic aorta; (2) abdominal aorta; (3) mesenteric artery; (4) renal artery; (5) iliac artery. A correlation was found ($r = 0.961$, $P < 0.05$), and the slope was 0.69 ± 0.12. Tissues were pretreated with chloroethylclonidine (10^{-4} M) for 60 min.

[^{125}I]HEAT binding study. The slope (0.23 ± 0.02, $r = 0.990$) obtained from the relationship between pD_2 value and the logarithm of the B_{max} value for untreated preparations reported by Takayanagi et al. (1991b) is significantly less than that (0.69 ± 0.12, $r = 0.961$) obtained for chloroethylclonidine-treated preparations in the present experiments, suggesting that these may be positive co-operativity by some different signal transduction pathways mediated through a different α_1-subtype. In functional studies using phenoxybenzamine, Oriowo et al. (1992) found that the regional differences in sensitivity of α_1-adrenoceptors of vascular smooth muscle for norepinephrine are due to receptor affinities. These results could suggest that some α_1-subtypes co-exist in rabbit arteries. Their findings support our observation that norepinephrine-induced contraction is caused by two pharmacologically distinct α_1-adrenoceptor subtypes in vascular smooth muscle of the rabbit. Moreover, the slope (0.69 ± 0.12, $r = 0.961$) we obtained from the relationship between the pD_2 value and the logarithm of B_{max} value differs significantly from unity, suggesting that the other subtypes such as α_{1D}-adrenoceptors, which are insensitive to chloroethylclonidine, may co-exist in these tissues.

From the above evidence, it can be concluded that the regional differences in potency (pD_2 value) of the α_1-adrenoceptor agonist, norepinephrine, are due to the differences in the population and density of α_1-adrenoceptor subtypes, and it also suggests the possibility that, in addition to α_{1A}- and α_{1B}-subtypes, other subtypes such as α_{1D} exist in the arteries of rabbit.

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