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Characterization of α_1-adrenoceptor-mediated contraction in the mouse thoracic aorta

Yoshihisa Yamamoto, Katsuo Koike *

Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan

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Abstract

In the mouse thoracic aorta, noradrenaline, adrenaline, phenylephrine and methoxamine behaved as full agonists. The pA_2 values for 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378) against each agonist were in good agreement with the generally accepted affinity value of α_{1D}-adrenoceptors. 5-Methylurapidil, 2-[2,6-dimethoxyphenoxyethyl]-aminomethyl-1,4-benzodioxane hydrochloride (WB 4101) and prazosin inhibited the contraction in response to noradrenaline. A significant correlation was obtained between the antagonist affinities in mouse thoracic aorta and those of native α_{1D}-adrenoceptors in rat thoracic aorta or with those of cloned α_{1d}-adrenoceptors, but not with those for either α_{1a}- or α_{1b}-adrenoceptors. Buspirone behaved as a partial agonist in mouse thoracic aorta, the contraction of which was antagonized by BMY 7378 with a pA_2 value (8.49) consistent with that found against noradrenaline (8.43). Clonidine acted as a partial agonist ($pD_2 = 5.94$). The pK_p value for clonidine against noradrenaline was similar to the pD_2 value for clonidine. The apparent pK_B value for BMY 7378 against clonidine was similar to the pA_2 value against other full agonists used in the present study. These results suggest that the α_{1D}-adrenoceptor subtype exists, and that the full agonists and the partial agonists evoke the contraction mediated through the α_{1D}-adrenoceptor in mouse thoracic aorta.

Keywords: α_{1D}-Adrenoceptor; Thoracic aorta, mouse; BMY 7378; Clonidine

1. Introduction

Pharmacological and molecular cloning studies have established operational and structural heterogeneity among the α_1-adrenoceptors (Minneman, 1988; Bylund et al., 1994; Ford et al., 1994). The α_1-adrenoceptor classification comprises three native subtypes, termed α_{1A}, α_{1B} and α_{1D}, and their cloned counterparts are now designated as α_{1a}, α_{1b} and α_{1d} (Bylund et al., 1994; Ford et al., 1994; Hieble et al., 1995b). Various groups have shown that the α_1-adrenoceptor antagonist, prazosin, does not discriminate between these subtypes (Ford et al., 1994; Hieble et al., 1995a,b; Michel et al., 1995). Functional pharmacological studies, however, have resulted in a subdivision of the α_1-adrenoceptors that is based on selectivity of prazosin. Muramatsu et al. (1990b, 1995) proposed that the α_1-adrenoceptors can be pharmacologically divided into α_{1H} and α_{1L} subtypes with high ($pA_2 > 9$) and low ($pA_2 < 9$) affinity for prazosin, respectively. The α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes are all included in the α_{1H}-adrenoceptor subtypes. There is a report that α_{1L}-adrenoceptor is a different conformation of α_{1A}-adrenoceptor (Ford et al., 1997).

The distribution of the various α_1-adrenoceptor subtypes is not homogeneous among the thoracic aorta of different animal species. For example, the contraction response seen in the thoracic aorta of the rat is mediated via the α_{1D}-adrenoceptor (Saussy et al., 1994; Kenny et al., 1995; Testa et al., 1995; Eltze, 1996), whereas in the guinea pig, the thoracic aorta responses are said to be mediated via α_{1L}-adrenoceptors (Muramatsu et al., 1990b; Yamamoto and Koike, 1999). On the other hand, α_{1A}- and α_{1B}- (Suzuki et al., 1990; Oriowo and Ruffolo, 1992; Castillo et al., 1993), α_{1B}- and α_{1L}- (Muramatsu et al., 1990a, 1998), α_{1D}- and α_{1L}- (Leonardi et al., 1997; Testa et al., 1997; Eltze et al., 1999) adrenoceptor-mediated contractions have been reported for rabbit aorta.

Targeted gene disruption has been used increasingly to elucidate the in vivo functions of several receptors, including some adrenoceptor subtypes (Link et al., 1995; Susulic

* Corresponding author. Tel.: +81-47-472-1419; fax: +81-47-472-1419.

E-mail address: ktkoike@phar.toho-u.ac.jp (K. Koike).

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et al., 1995; MacMillan et al., 1996; Rohrer et al., 1996; Cavalli et al., 1997). The potential functional changes occurring in knockout mice might allow, on one hand, to assign distinct functions to the receptor that has been deleted, and on the other, to test the functional redundancy among receptor subtypes. However, there is little information about the distribution of the α_1-adrenoceptor subtype in the normal mouse.

BMY 7378 is the first selective antagonist at the α_{1D}-adrenoceptor subtype (Saussy et al., 1994; Goetz et al., 1995). This agent is used with various tissues to detect the presence of the α_{1D}-adrenoceptor subtype. Recently, Hussain and Marshall (1997, 2000) reported that the pA_2 value for BMY 7378 against phenylephrine was inconsistent with the pK_B value for BMY 7378 against methoxamine in the rat mesenteric artery in which there is an α_{1D}-adrenoceptor subtype mediating contraction.

Therefore in the present study, we tried to determine (1) which α_1-adrenoceptor subtypes are involved in the mouse thoracic aorta and (2) whether the functional subtypes mediating adrenergic contraction are dependent on the agonists, by calculating the pA_2 values of some antagonists.

2. Materials and methods

2.1. Mechanical responses

Male albino ddY mice (20–30 g) were killed by a blow on the head and the thoracic aorta was isolated and dissected free of excess fat and connective tissues. The intimal surface of the thoracic aorta was gently rubbed with a polyethylene tube to remove the endothelium. Functional loss of endothelial cells was confirmed by the loss of the relaxation response to acetylcholine (1 μM). The aorta was cut into 4-mm ring segments. Each ring segment was suspended in a 20-ml organ bath filled with a Ringer–Locke solution (154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 2.1 mM $MgCl_2$, 5.9 mM $NaHCO_3$ and 2.8 mM glucose) kept at 37 °C and bubbled with a mixture of 95% O_2 and 5% CO_2. Tension was monitored continuously and recorded isometrically by a force-displacement transducer. Experiments were conducted in the presence of propranolol (10 μM), yohimbine (300 nM), desmethylimipramine (100 nM) and normetanephrine (1 μM) to block β-adrenoceptors and α_2-adrenoceptors and to inhibit neural and non-neural uptake of noradrenaline, respectively. The strips were allowed to equilibrate for 90 min, were then contracted with noradrenaline (100 nM), and allowed to equilibrate for 30 min after washout. This was repeated until two successive contractions of approximately equal sizes had been obtained.

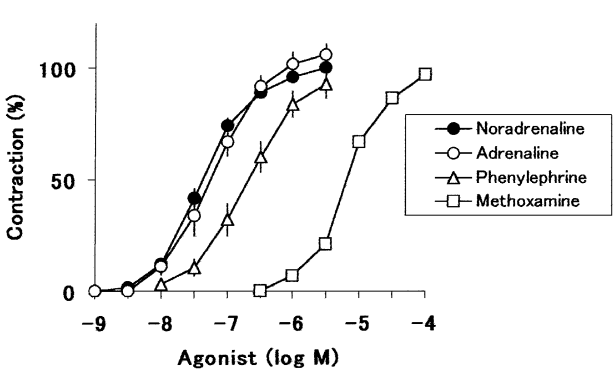

Fig. 1. Contractile effects of α_1-adrenoceptor agonists in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (M) of agonists. Each point is the mean ± S.E. of six to eight experiments.

Table 1
Contraction parameters for various agonists in the mouse thoracic aorta

Agonist	pD_2 value	Intrinsic activity
Noradrenaline	7.47 ± 0.06	1
Adrenaline	7.23 ± 0.09	1.06 ± 0.05
Phenylephrine	6.72 ± 0.08	0.93 ± 0.06
Methoxamine	5.18 ± 0.06	0.98 ± 0.07
Clonidine	5.94 ± 0.09	0.22 ± 0.03
Buspirone	7.51 ± 0.09	0.56 ± 0.03

Each value indicates the mean ± S.E. of four to eight experiments.

The competitive antagonistic activities were expressed as pA_2 values (negative logarithms of dissociation constant). The concentration–response curves for agonists were obtained cumulatively. Contraction was expressed as a percentage of the maximal response produced by the agonists. After determination of control concentration–response curves, the strips were equilibrated with a competitive antagonist for 15 min. Concentration–response curves were then obtained in the presence of the antagonist and the procedure was repeated with a high (threefold) concentration of the antagonist in the same preparation. After determination of the control concentration–response curves, two or three successive cumulative concentration–response curves for agonists were determined. The curves were nearly superimposable and changes in sensitivity, sensitization, or desensitization were minimal (data not shown). The pA_2 values were calculated according to a method (Tallarida et al., 1979), which was originally described by Arunlakshana and Schild (1959).

The antagonistic potency of clonidine was also measured against noradrenaline with the same protocol as described above. The negative logarithm of the dissociation constant of an antagonist was estimated by the method of Lemoine and Kaumann (1982) and also expressed as pK_p value.

In one experiment, the concentration–response curve for the noradrenaline declined in the presence of prazosin in the mouse thoracic aorta. This was thought to indicate a hemi-equilibrium state between agonist, antagonist and receptors as reported by Paton and Rang (1966), and Paton

Table 2
The pA_2 values of BMY 7378 against different agonists in the mouse thoracic aorta

Agonist	pA_2 value	Slope
Noradrenaline	8.43±0.08	1.02±0.05
Adrenaline	8.37±0.08	1.09±0.05
Phenylephrine	8.45±0.09	1.03±0.04
Methoxamine	8.38±0.14	1.00±0.10
Buspirone	8.49±0.24	1.08±0.13

Each value indicates the mean±S.E. of four to eight experiments.

and Waud (1967). An estimate of the dissociation constant (K_B) of the antagonist was made using Eq. (1) (Paton and Rang, 1966),

$$1/[A] = 1/K_A \cdot \rho/(1-\rho) + 1/(1-\rho) \cdot 1/[A'] \quad (1)$$

where [A] and [A′] refer to the equieffective concentrations of the agonist in the absence and presence of the antagonist (B), and K_A is the dissociation constant of the agonist. Furthermore, ρ in Eq. (1) is the fractional receptor occupancy, which is obtained from Gaddum's equation (Eq. (2)),

$$\rho = 1/\{1 + K_A/[A](1 + [B]/K_B)\} \quad (2)$$

wherein [A] and [B] are concentrations of the agonist and the antagonist, respectively, and K_A and K_B refer to their respective equilibrium dissociation constants.

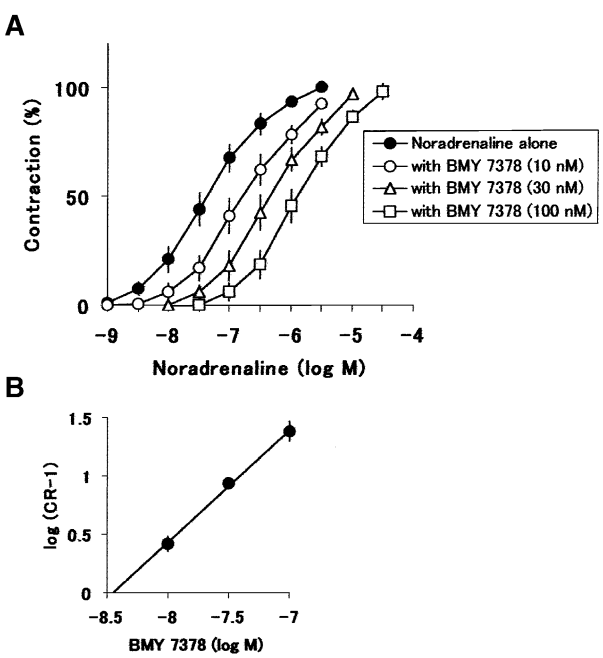

Fig. 2. (A) Effects of BMY 7378 on noradrenaline-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (M) of noradrenaline. (B) Schild plot for antagonism of noradrenaline by BMY 7378. Ordinate: logarithm of equieffective concentration ratio (CR) of noradrenaline −1. Abscissa: log concentration (M) of BMY 7378. Each point is the mean±S.E. of six experiments.

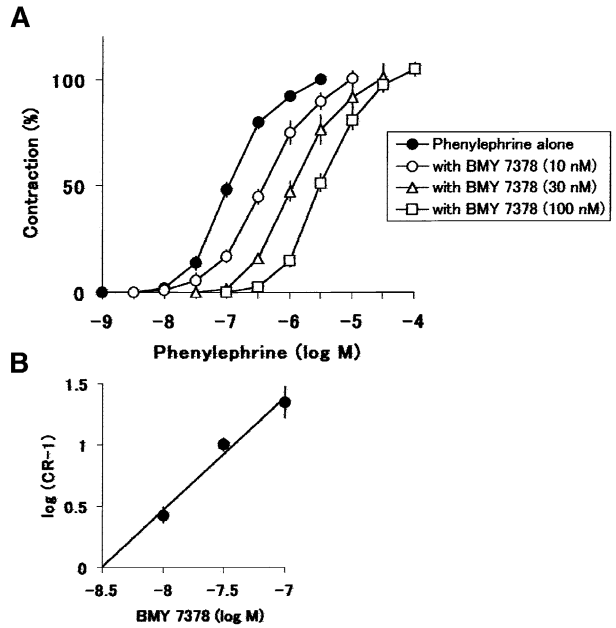

Fig. 3. (A) Effects of BMY 7378 on phenylephrine-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by phenylephrine (3 μM). Abscissa: log concentration (M) of phenylephrine. (B) Schild plot for antagonism of phenylephrine by BMY 7378. Ordinate: logarithm of equieffective concentration ratio (CR) of phenylephrine −1. Abscissa: log concentration (M) of BMY 7378. Each point is the mean±S.E. of six experiments.

Therefore, a double reciprocal regression of $1/[A]$ upon $1/[A']$ should yield a straight line with a positive intercept, according to Eq. (1). The K_B then can be calculated from Eq. (3).

$$K_B = [B]/(\text{slope} - 1). \quad (3)$$

In one experiment, we used tissue treated with an irreversible α_{1B}-adrenoceptor alkylating agent chloroethylclonidine. After determination of the control concentration–response curves, the tissue was treated with 100 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. Chloroethylclonidine was removed from the nutrient solution by repeated washings after treatment of the tissue with the agent for 60 min.

Table 3
The pA_2 values of α_1-adrenoceptor antagonists against noradrenaline in the mouse thoracic aorta

Antagonist	pA_2 value	Slope
Prazosin	9.71±0.06[a]	–
WB 4101	9.62±0.06	0.93±0.04
5-Methylurapidil	7.49±0.11	1.07±0.08
BMY 7378	8.43±0.08	1.02±0.05

Each value indicates the mean±S.E. of four to eight experiments.
[a] Is the negative logarithm of the dissociation constant, K_B value, calculated from Eq. (1).

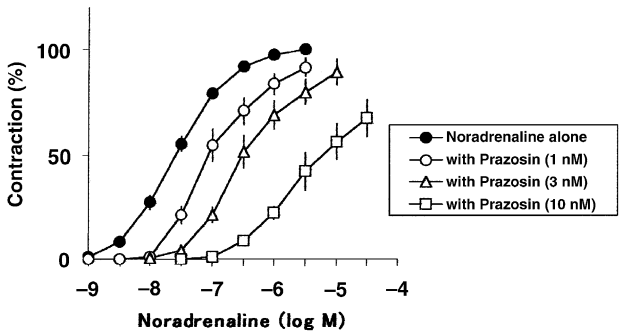

Fig. 4. Effects of prazosin on noradrenaline-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (*M*) of noradrenaline.

2.2. Data analysis

Numerical results were expressed as means ± S.E. and statistical analyses were performed using Student's *t*-test and Dunnet's multiple range test as appropriate. A *P* value of less than 0.05 was considered significant.

2.3. Drugs

The following drugs were used: (−)-noradrenaline bitartrate, phenylephrine hydrochloride (Wako-Junyaku, Osaka, Japan); 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378), buspirone hydrochloride, chloroethylclonidine dihydrochloride, 5-methylurapidil and 2-[2,6-dimethoxyphenoxyethyl]aminomethyl-1,4-benzodioxane hydrochloride (WB 4101) (Research Biochemicals, Natick, MA); (−)-adrenaline bitartrate, clonidine (2-[2,6-dichloroaniline]-2-imidazoline) hydrochloride, desmethylimipramine hydrochloride, methoxamine (α-[1-aminoethyl]-2,5-dimethoxybenzylalchol) hydrochloride, (±)-normetanephrine hydrochloride, (±)-propranolol hydrochloride, prazosin hydrochloride and yohimbine hydrochloride (Sigma, St. Louis, MO).

5-Methylurapidil was dissolved in dimethylsulfoxide (DMSO) at the initial concentration of 2 mM, and diluted in distilled water. All other drugs were dissolved in distilled water.

3. Results

3.1. Effect of agonists in the mouse thoracic aorta

In the mouse thoracic aorta, noradrenaline, adrenaline, phenylephrine and methoxamine evoked contraction in a concentration-dependent manner. Each agonist yielded the full agonistic action, the rank order of potency being nor-

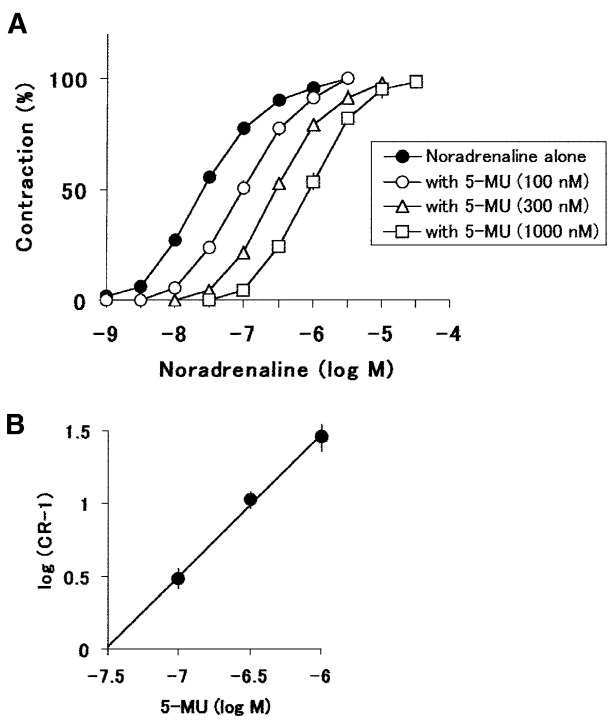

Fig. 5. (A) Effects of 5-methylurapidil (5-MU) on noradrenaline-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (*M*) of noradrenaline. (B) Schild plot for antagonism of noradrenaline by 5-MU. Ordinate: logarithm of equieffective concentration ratio (CR) of noradrenaline −1. Abscissa: log concentration (*M*) of 5-MU. Each point is the mean ± S.E. of six experiments.

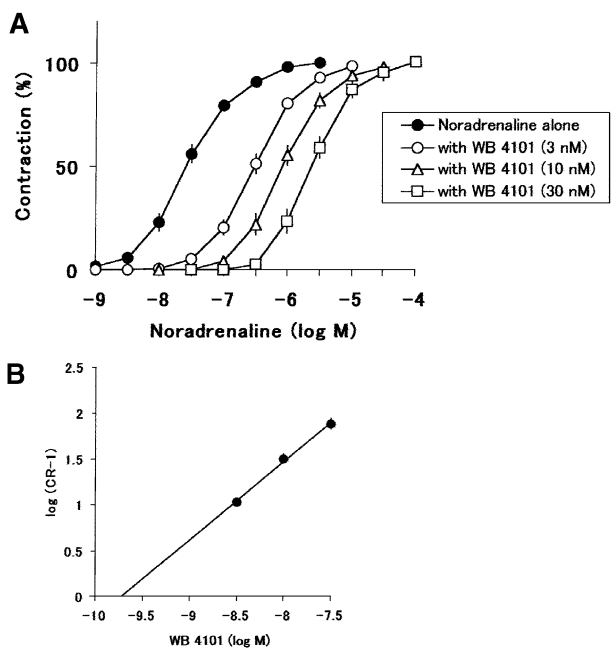

Fig. 6. (A) Effects of WB 4101 on noradrenaline-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (*M*) of noradrenaline. (B) Schild plot for antagonism of noradrenaline by WB 4101. Ordinate: logarithm of equieffective concentration ratio (CR) of noradrenaline −1. Abscissa: log concentration (*M*) of WB 4101. Each point is the mean ± S.E. of six experiments.

adrenaline = adrenaline > phenylephrine > methoxamine (Fig. 1). The pD_2 values and intrinsic activities for the agonists are summarized in Table 1.

3.2. Effect of BMY 7378 on the contraction induced by agonists

The responses to agonists (noradrenaline, adrenaline, phenylephrine and methoxamine) were antagonized by BMY 7378 in a concentration-dependent manner. Schild regression analyses carried out for BMY 7378 against each agonist gave pA_2 values of approximately 8.4. The slopes of the Schild regression lines were not significantly different from unity (Table 2; Figs. 2 and 3).

3.3. Effect of prazosin, 5-methylurapidil and WB 4101 on the contraction induced by noradrenaline

The response to noradrenaline was antagonized by prazosin in a concentration-dependent manner. Prazosin shifted to the right the concentration–response curve for noradrenaline. The negative logarithm of the dissociation constant, pK_B of prazosin calculated by Eq. (1) of Paton and Rang (1966) was 9.71 (Table 3; Fig. 4).

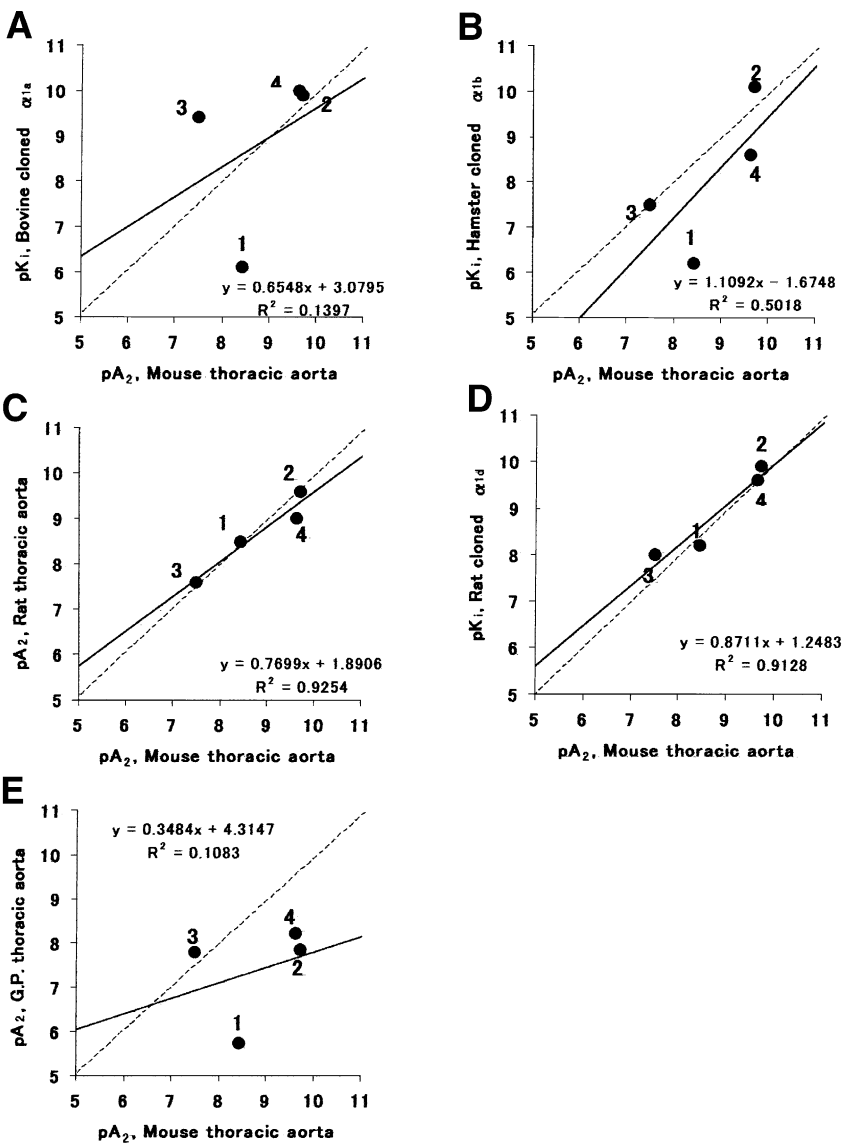

Fig. 7. Correlation plots showing the relationship of affinity estimates from in vitro functional analysis of mouse thoracic aorta for some antagonists (pA_2) compared with (A) bovine cloned α_{1a}-adrenoceptor (pK_i), (B) hamster cloned α_{1b}-adrenoceptors (pK_i), (C) rat thoracic aorta (α_{1D}-adrenoceptor, pA_2), (D) rat cloned α_{1d}-adrenoceptor (pK_i) (Ford et al., 1996) and (E) guinea pig thoracic aorta (α_{1L}-adrenoceptor, pA_2) (Yamamoto and Koike, 1999). Cloned mammalian α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors expressed in rat-1 fibroblasts (Ford et al., 1996). The solid lines were obtained by linear regression, the dashed lines represent the line of identity. The num(bers in each figure indicate the antagonists (1: BMY 7378; 2: prazosin; 3: 5-methylurapidil; and 4: WB 4101).

The response to noradrenaline was antagonized by 5-methylurapidil and WB 4101 in a concentration-dependent manner. Schild regression analyses carried out for 5-methylurapidil and WB 4101 against noradrenaline gave pA_2 values of 7.49 and 9.62, respectively. The slopes of the regression lines were not significantly different from unity (Table 3; Figs. 5 and 6).

3.4. Correlation coefficients between mouse thoracic aorta, and native or cloned α1-adrenoceptor subtypes

We studied the correlations between the pA_2 or pK_B values obtained in the present study with mouse thoracic aorta (Table 3), and pK_i values for the displacement of [^{3}H] prazosin or pA_2 values obtained from the functional analysis with the α_1-adrenoceptor subtypes (Ford et al., 1996; Yamamoto and Koike, 1999). Only those antagonists (BMY 7378, prazosin, 5-methylurapidil and WB 4101), which were used both by us and in previous reports (Ford et al., 1996; Yamamoto and Koike, 1999), were included in the analysis.

We obtained a good correlation for the pA_2 values reported for rat thoracic aorta (α_{1D}-adrenoceptor) and pK_i values reported for rat cloned α_{1d}-adrenoceptor with the pA_2 values estimated with mouse thoracic aorta (R^2 values were 0.93 and 0.92, respectively), and the regression lines were close to the line of identity (Fig. 7). In contrast, we did not observe any significant correlation for the mouse thoracic aorta with the pK_i values reported for cloned bovine α_{1a}-, hamster α_{1b}-adrenoceptors and the pA_2 values reported for guinea pig thoracic aorta (α_{1L}-adrenoceptor). The correlation coefficients (R^2 values) were 0.14, 0.51 and 0.11, respectively (Fig. 7).

3.5. Effect of chloroethylclonidine treatment on the contraction induced by noradrenaline

In tissue treated for 60 min with the irreversible α_{1B}-adrenoceptor alkylating agent, chloroethylclonidine (100 μM), the concentration–response curve for noradrenaline was shifted approximately 100-fold to the right (Fig. 8).

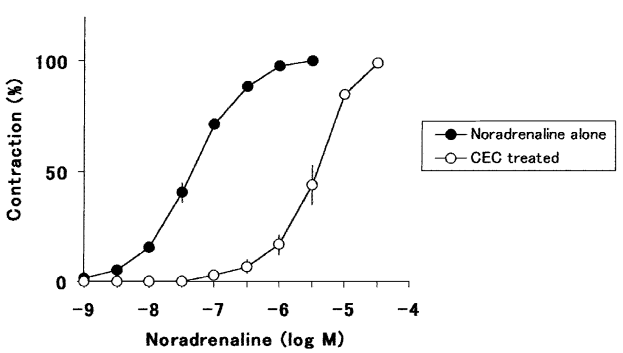

Fig. 8. Effects of treatment with chloroethylclonidine (CEC: 100 μM, 60 min) on noradrenaline-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (M) of noradrenaline.

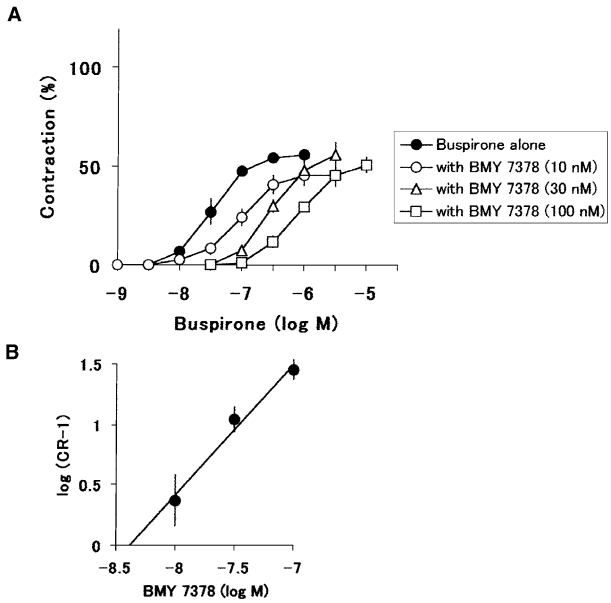

Fig. 9. (A) Effects of BMY 7378 on buspirone-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (M) of buspirone. (B) Schild plot for antagonism of buspirone by BMY 7378. Ordinate: logarithm of equieffective concentration ratio (CR) of buspirone −1. Abscissa: log concentration (M) of BMY 7378. Each point is the mean ± S.E. of five experiments.

3.6. The effect of buspirone in the mouse thoracic aorta

Buspirone acted as a partial agonist (Table 1). The response to buspirone was antagonized by BMY 7378 in a concentration-dependent manner. Schild regression analysis carried out for BMY 7378 against buspirone gave a pA_2 value of 8.49. The slope of the regression line was not significantly different from unity (Table 2; Fig. 9).

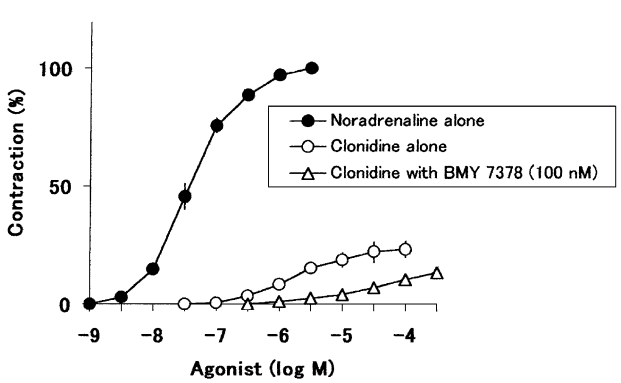

Fig. 10. Effects of BMY 7378 on clonidine-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (M) of clonidine or noradrenaline.

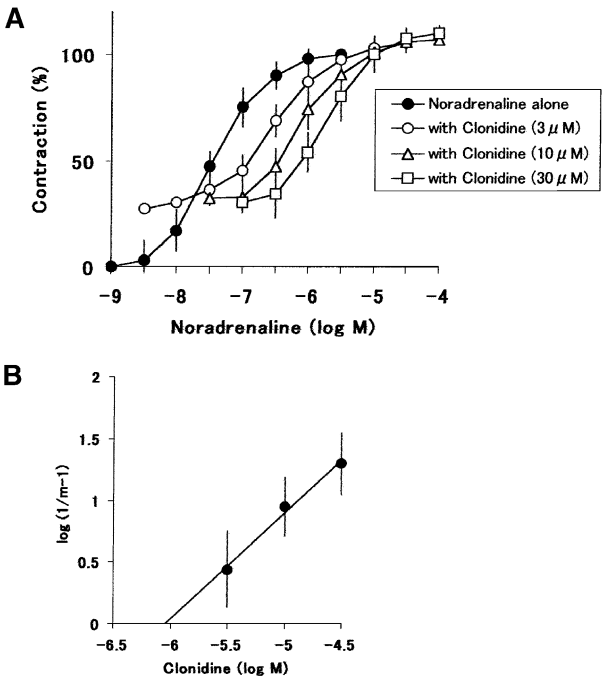

Fig. 11. (A) Effects of clonidine on noradrenaline-induced contraction in the mouse thoracic aorta. Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (3 μM). Abscissa: log concentration (M) of noradrenaline. (B) The concentration-dependence of the blocking action for a partial agonist, clonidine, in a double-log plot including the classical Schild plot as a special case. Ordinate: logarithm of $1/m-1$ (see Section 3). Abscissa: log concentration (M) of clonidine. Each point is the mean ± S.E. of four experiments.

3.7. The effects of clonidine in the mouse thoracic aorta

Clonidine acted as a partial agonist in the mouse thoracic aorta (Table 1; Fig. 10). The drug contracted the preparation and acted as a synergist with low concentrations of noradrenaline but competitively inhibited the contractile responses to higher concentrations of noradrenaline (Fig. 11). After the slope m of the weighted regression of equieffective concentrations of noradrenaline in the absence and presence of a concentration [P] of the partial agonist, clonidine, had been estimated (Lemoine and Kaumann, 1982), the regression of $\log(1/m-1)$ against log[P] was shown to be linear with a slope near unity (0.86 ± 0.09, not significantly different from 1.00; $P > 0.05$). The intercept representing the negative log of the dissociation constant (pK_p value) of clonidine amounted to 6.16 ± 0.40 (Fig. 11). The pD_2 value and intrinsic activity for clonidine are summarized in Table 1. The contractile response to clonidine was antagonized by BMY 7378 (100 nM), the apparent pK_B value was 8.87 ± 0.18 (Fig. 10).

4. Discussion

We examined the effects of BMY 7378, the first selective α_{1D}-adrenoceptor antagonist (Saussy et al., 1994; Goetz et al., 1995), on contractions induced by some agonists (noradrenaline, adrenaline, phenylephrine and methoxamine) in the mouse thoracic aorta. The four agonists used in the present study evoked contraction in a concentration-dependent manner, and intrinsic activities were not significantly different from one, suggesting full agonism (Table 1; Fig. 1). The rightward shifts of the concentration–response curves for agonists were observed with 10–100 nM BMY 7378 (Figs. 2 and 3 about noradrenaline- and phenylephrine-induced contraction). The pA_2 values for BMY 7378 against the agonists from the Schild plot were similar to each other and were in good agreement with the generally accepted value for rat thoracic aorta ($pA_2 = 8.5$, Ford et al., 1996). The slopes of the Schild regression lines were not significantly different from unity (Table 2), suggesting a simple competitive antagonism against each agonist. These results indicate that the contractile responses to agonists are mediated through an identical site and that the α_{1D}-adrenoceptor subtype predominates in the mouse thoracic aorta. We reported a pA_2 value of 5.73 for BMY 7378 with guinea pig thoracic aorta (Yamamoto and Koike, 1999). Therefore, the α_1-adrenoceptor mediating contraction of mouse thoracic aorta is similar pharmacologically to the α_1-adrenoceptor subtype in rat thoracic aorta, but not in guinea pig. We tried to estimate the pA_2 values for the α_1-adrenoceptor antagonists, 5-methylurapidil, WB 4101 and prazosin, on noradrenaline-induced contraction. The concentration–response curves for noradrenaline were shifted to the right by the α_{1A}-adrenoceptor antagonists, 100–1000 nM 5-methylurapidil or 3–30 nM WB 4101, respectively (Figs. 5 and 6). Neither slope of the Schild regression lines was significantly different from unity, suggesting a simple competitive antagonism (Table 3). The pA_2 values for 5-methylurapidil and WB 4101 were 7.49 and 9.62, respectively, which is similar to the value for rat thoracic aorta ($pA_2 = 7.6$ and 9.0, Ford et al., 1996). The pA_2 value found for 5-methylurapidil at the mouse thoracic α_1-adrenoceptors was significantly lower than the value previously reported for its affinity at the α_{1A}-adrenoceptors (pA_2 or pK_i value = about 9–10, Aboud et al., 1993; Burt et al., 1995; Ford et al., 1996; Hussain and Marshall, 1997). The low potency of 5-methylurapidil indicates that the α_{1A}-adrenoceptor plays no significant role in the contractile responses of mouse thoracic aorta to noradrenaline. On the other hand, WB 4101 displayed a high affinity (9.62). Ford et al. (1996) reported that WB 4101 displayed a high affinity at both α_{1A}- and α_{1D}-adrenoceptor subtypes. Therefore, it is suggested that the antagonism with WB 4101 may be a block of the α_{1D}-adrenoceptors, but not of the α_{1A}-adrenoceptors. As shown in Fig. 4, prazosin shifted the concentration–response curve for noradrenaline to the right and depressed the maximum response. This phenomenon can be interpreted as involving a hemi-equilibrium (Paton and Rang, 1966; Paton and Waud, 1967; Kenakin, 1984). It is well known that in the hemi-equilibrium state, the antagonist behaves as an essen-

tially irreversible blocker and produces unsurmountable antagonism. The depression of the maximal response for any given dose ratio depends on the efficacy of the agonist (Paton and Rang, 1966; Paton and Waud, 1967; Kenakin, 1984). According to Paton's theory (Paton, 1961), the rate constant for the dissociation of a potent competitive antagonist is smaller than that of a less potent antagonist, that is, the potent antagonist forms a complex with the receptor which is slowly broken up. Prazosin is thought to dissociate more slowly than other antagonists from the α_1-adrenoceptors in the mouse thoracic aorta. Therefore, the hemiequilibrium state was observed in the experiments with prazosin but not in those with BMY7378, 5-methylurapidil or WB 4101. The dissociation constant (pK_B) for prazosin, using Eq. (1) was 9.71, which was also similar to the pA_2 value with rat thoracic aorta ($pA_2 = 9.6$, Ford et al., 1996). A good correlation was obtained for the affinity values reported for α_{1D}- and α_{1d}-adrenoceptors, but not for α_{1a}-, α_{1b}- and α_{1L}-adrenoceptors with the pA_2 or pK_B values estimated in mouse thoracic aorta. Moreover, the regression lines were close to the line of identity (Fig. 7). These results strongly suggest that the α_1-adenoceptor mediating contraction of the mouse thoracic aorta is similar pharmacologically to the putative α_{1D}-adrenoceptor subtype in the rat thoracic aorta.

Recently, Eltze et al. (1999) reported that the 5-HT_{1A} receptor agonist, buspirone, which differs only in its arylpiperazinyl moiety from its analog, BMY 7378, was a weak antagonist without intrinsic activity at the α_{1A}-, α_{1B}- and α_{1L}-adrenoceptors, but behaved as a partial agonist in rat aorta used as model for the α_{1D}-adrenoceptors. In the present study, we tried to test the effects of buspirone in mouse thoracic aorta. Buspirone evoked contraction concentration dependently, and the intrinsic activity was 0.56, suggesting partial agonism (Table 1). The rightward shift of the concentration–response curve for buspirone was also observed with 10–100 nM BMY 7378 (Fig. 9), as seen for the noradrenaline-induced contraction (Fig. 2). The pA_2 value for BMY 7378 calculated from the Schild plot (8.49) is in good agreement with the value against noradrenaline (Table 2). These results suggest that buspirone and noradrenaline evoke contraction mediated through an identical site (e.g. α_{1D}-adrenoceptor subtype). Based on these results, it can be confirmed that buspirone behaves as a partial agonist to the α_{1D}-adrenoceptor subtype in the mouse thoracic aorta, as reported for the rat thoracic aorta (Eltze et al., 1999).

It is well known that the partial agonists have little receptor reserve, while there is a large receptor reserve for the full agonists (Stephenson, 1956; Van Rossum and Ariëns, 1962). If the partial agonists interacted with one recognition site in the receptors, the pD_2 values of the partial agonists would be equal to their pA_2 values or pK_p values (Takayanagi et al., 1984a,b). In the present study, the pD_2 value of clonidine was nearly identical to its pK_p value in the mouse thoracic aorta, suggesting that clonidine interacted with one recognition site. Moreover, the rightward shift of the clonidine concentration–response curve was also observed with 100 nM BMY 7378 (Fig. 10), and the apparent pK_B value for BMY 7378 was in good agreement with the pA_2 values against the full agonists used in the present study. These results indicate that the contractile response to clonidine is also mediated through the α_{1D}-adrenoceptor subtype as it is to that of the full agonists in the mouse thoracic aorta. Satoh et al. (1998) reported that the α_{1D}-adrenoceptor subtype was activated by the full agonist, phenylephrine, but not by the partial agonist, tizanidine in the rabbit iliac artery. On the other hand, Iwanaga et al. (1998) reported that clonidine evoked a contraction mediated by the α_1-adrenoceptor in the rat thoracic aorta, which has been reported to be supplied with α_{1D}-adrenoceptors (Saussy et al., 1994; Kenny et al., 1995; Testa et al., 1995; Eltze, 1996), indicating α_{1D}-adrenoceptor activation by clonidine. Based on our results, it is suggested that only, or mainly, in α_{1D}-adrenoceptor contributing tissue (probably involving the mouse thoracic aorta) may the partial agonist activate the α_{1D}-adrenoceptor.

Recently, Cavalli et al. (1997) have shown that the response to phenylephrine is attenuated in aorta from α_{1B}-adrenoceptor knockout mice, and suggested the participation of the α_{1B}-adrenoceptor to the contraction of mouse aorta. However, the concentration–response curve for phenylephrine in α_{1B}-adrenoceptor deficient $-/-$ mice is only threefold shifted to the right compared to the curve for phenylephrine in α_{1B}-adrenoceptor $+/+$ mice, and about 75% of the maximal contraction remained in α_{1B}-adrenoceptor $-/-$ mice. From these results, we consider that phenylephrine evokes a contraction mediated partly, but not mainly, through α_{1B}-adrenoceptor in the mouse thoracic aorta. In the present study, the concentration–response curve for noradrenaline was shifted to the right by treatment with the irreversible α_{1B}-adrenoceptor alkylating agent, chloroethylclonidine (Fig. 8), which suggests that the α_{1B}-adrenoceptor participates in the contraction of mouse thoracic aorta. However, the rightward shift of the concentration–response curve is more marked than that reported for α_{1B}-adrenoceptor knockout mice (Cavalli et al., 1997). A number of studies have shown that this agent inactivates all three α_1-adrenoceptor subtypes, albeit to different degrees (Forray et al., 1994; Hatano et al., 1994; Laz et al., 1994). Therefore, it is suggested that chloroethylclonidine inactivated not only the α_{1B}-adrenoceptors but also probably the α_{1D}-adrenoceptors in the mouse thoracic aorta. It is possible that multiple α_1-adrenoceptor subtypes are involved in the contraction of the mouse thoracic aorta as reported for rat thoracic aorta (Van der Graaf et al., 1996). We suggest that mainly the α_{1D}-adrenoceptor contributes to and participates in the contraction of the mouse thoracic aorta, to judge from our results.

In conclusion, the present study indicated that (1) mainly the α_{1D}-adrenoceptor subtypes participate in contraction in

the mouse thoracic aorta and that (2) the contractile responses to full agonists (noradrenaline, adrenaline, phenylephrine and methoxamine) and partial agonists (buspirone and clonidine) are mediated through an identical site, the α_{1D}-adrenoceptor, in the mouse thoracic aorta.

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