JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 103–108

Received March 9, 2000
Accepted August 2, 2000
ISSN 0022-3573

Antagonistic effects of selective α_1-adrenoceptor antagonists MDL73005EF and tamsulosin and partial agonists clonidine and tizanidine in rat thoracic aorta and rabbit iliac artery

Mitsutoshi Satoh, Keisuke Enomoto and Katsuo Koike

Abstract

The antagonistic effects of MDL73005EF and tamsulosin and partial agonists clonidine and tizanidine at rat thoracic aorta and rabbit iliac artery α_1-adrenoceptors were investigated in this study.

Selective α_1-adrenoceptor antagonists MDL73005EF and tamsulosin dose-dependently shifted the concentration–response curves for noradrenaline to the right. Schild plots of the results obtained from the inhibition by MDL73005EF (pA_2 8.30 ± 0.04) and tamsulosin (pA_2 10.51 ± 0.06) of noradrenaline yielded a straight line with a slope of unity in rat thoracic aorta. The slopes of Schild plots obtained from the inhibition by MDL73005EF and tamsulosin of noradrenaline were significantly different from unity in rabbit iliac artery. Schild plots of the results obtained from the inhibition by clonidine and tizanidine of noradrenaline yielded a straight line with a slope of unity in rat thoracic aorta (pA_2 7.08 ± 0.04 and 7.32 ± 0.04, respectively).

These results suggest that α_{1D}-adrenoceptors play a significant role in the α_1-adrenoceptor-agonist-induced contraction of rat thoracic aorta and rabbit iliac artery, and that clonidine and tizanidine interact with the α_{1D}-adrenoceptor subtype as competitive antagonists in rat thoracic aorta.

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

Mitsutoshi Satoh, Keisuke Enomoto, Katsuo Koike

Correspondence: M. Satoh, Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan

Funding: This study was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No.11672191).

Introduction

Pharmacological studies have classified the α_1-adrenoceptors into three major subtypes, α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, for native receptors. The α_{1D}-adrenoceptor subtype plays an important role in noradrenaline-induced muscle contraction in rat aorta using the selective α_{1D}-adrenoceptor antagonist BMY 7378 (Saussy et al 1994; Kenny et al 1995). Rabbit renal and iliac artery were contracted via the activation of the α_{1D}-adrenoceptor subtype in addition to the α_{1A}- and α_{1B}-adrenoceptor subtypes, whereas rabbit thoracic and abdominal aorta were contracted via the activation of α_{1A}- and α_{1B}-adrenoceptor subtypes using BMY 7378, WB4101 and 5-methylurapidil (Satoh et al 1998, 1999). Putative α_1-adrenoceptor antagonist MDL73005EF apparently has a sensitivity over 10-fold higher for the α_{1D}-adrenoceptor subtype ($pK_B \sim 8$) than for α_{1A}- and α_{1B}-adrenoceptor subtypes ($pK_B \sim 7$) (Hieble et al 1995). It is also reported that tamsulosin is a high affinity antagonist for α_{1A}- and α_{1D}-adrenoceptors, and possesses a higher affinity ($pK_B \sim 10$) for α_{1A}- and α_{1D}-adrenoceptor subtypes than for the α_{1B}-adrenoceptor subtype

($pK_B \sim 9$) (Noble et al 1997). Full agonists, such as phenylephrine and noradrenaline, produce a muscle contraction through α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes. Partial agonists, such as tizanidine and clonidine, produce a muscle contraction only through the α_{1A}-adrenoceptor subtype in rabbit thoracic aorta and iliac arteries (Takayanagi et al 1991; Satoh et al 1992a, b, 1998). Partial agonists do however interact with the α_{1A}-and α_{1B}-adrenoceptor subtype as obtained in [^{3}H]prazosin binding experiments (Satoh et al 1992a, b). In rabbit iris dilator, which contracts through the activation of α_{1B}-adrenoceptors, partial agonists act as competitive α_{1B}-adrenoceptor antagonists (Takayanagi et al 1984, 1992).

To clarify the antagonistic effects of MDL73005EF and tamsulosin in rabbit iliac artery, in which the α_{1D}-adrenoceptor subtype co-exists with α_{1A}- and α_{1B}-subtypes, and to clarify the effects of partial agonists clonidine and tizanidine on the α_{1D}-adrenoceptor subtype, we studied the contractile responses to noradrenaline in rat thoracic aorta and rabbit iliac artery.

Materials and Methods

Drugs

The following drugs were used: MDL73005EF (8-[2-(1,4-benzodioxan-2-ylmethylamino)ethyl]-8-azaspiro[4,5]decane-7,9-dione hydrochloride; Research Biochemicals Inc., Natick, MA); (−)-tamsulosin hydrochloride ((−)-(*R*)-5-[2-[[2-(*o*-ethoxyphenoxy)-ethyl]amino]propyl]-2-methoxybenzenesulphonamide hydrochloride) (Yamanouchi, Tokyo, Japan); L-noradrenaline bitartrate (Wako-Junyaku, Osaka, Japan); clonidine hydrochloride (Nakalai, Kyoto, Japan); tizanidine hydrochloride (Sandoz, Tokyo, Japan); desmethylimipramine hydrochloride, (±)-normetanephrine hydrochloride, (±)-propranolol hydrochloride and yohimbine hydrochloride (Sigma, St Louis, MO), all in powder form; pentobarbital sodium (Abbott Lab., North Chicago, IL). Other chemicals used were of analytical grade.

Mechanical responses

Male Wistar rats, 250–350 g, and male albino rabbits, 2.0–3.0 kg, were anaesthetized with an intravenous injection of pentobarbital sodium (50 mg kg^{-1}) and killed by bleeding from the carotid arteries. Rat thoracic aorta and rabbit iliac arteries were quickly removed and a segment was cut into a helical strip. Endothelial cells were removed by 20 gentle rubbings of the luminal surface with a cotton probe. Strips were about 10 mm long and 2 mm wide. Pieces of tissue were mounted in glass organ baths containing 20 mL of physiological salt solution (PSS) of the following composition (mM): 118 NaCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$ and 11.0 glucose (pH = 7.4 at 37°C). Each bath was continuously gassed with a mixture of 95 % O_2 and 5 % CO_2. The solution contained propranolol (10^{-6} M), yohimbine (3×10^{-7} M), desmethylimipramine (10^{-7} M) and normetanephrine (10^{-6} M) to block α-adrenoceptors and α_2-adrenoceptors and to inhibit neural and non-neural uptake of catecholamines, respectively. Responses to drugs were isometrically recorded under a resting tension of 1.0 g for rat thoracic aorta and 0.5 g for rabbit iliac artery. The strips were allowed to equilibrate for 90 min. They were contracted with noradrenaline (10^{-6} M) and allowed to equilibrate for 30 min after wash-out. This was repeated until two successive contractions of approximately equal size had been obtained. After determination of control concentration–response curves obtained from the cumulative application of the agonist, the strips were equilibrated with a competitive antagonist for 30 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 in the same preparation. The curves were nearly superimposable and changes in sensitivity, sensitization or desensitization were minimal. The competitive antagonistic activities were expressed as pA_2 values (negative logarithms of the dissociation constant). The pA_2 values were calculated according to the method of Arunlakshana & Schild (1959).

Statistics

Numerical results are expressed as means ± s.e.m., and statistical significance was calculated with Student's *t*-test. $P < 0.05$ was considered to indicate a significant difference.

Results

Effects of MDL73005EF and tamsulosin

Noradrenaline produced concentration-dependent contractions of rat thoracic aorta and rabbit iliac artery with pD_2 values of 8.28 ± 0.02 (n = 4) and 7.94 ± 0.13

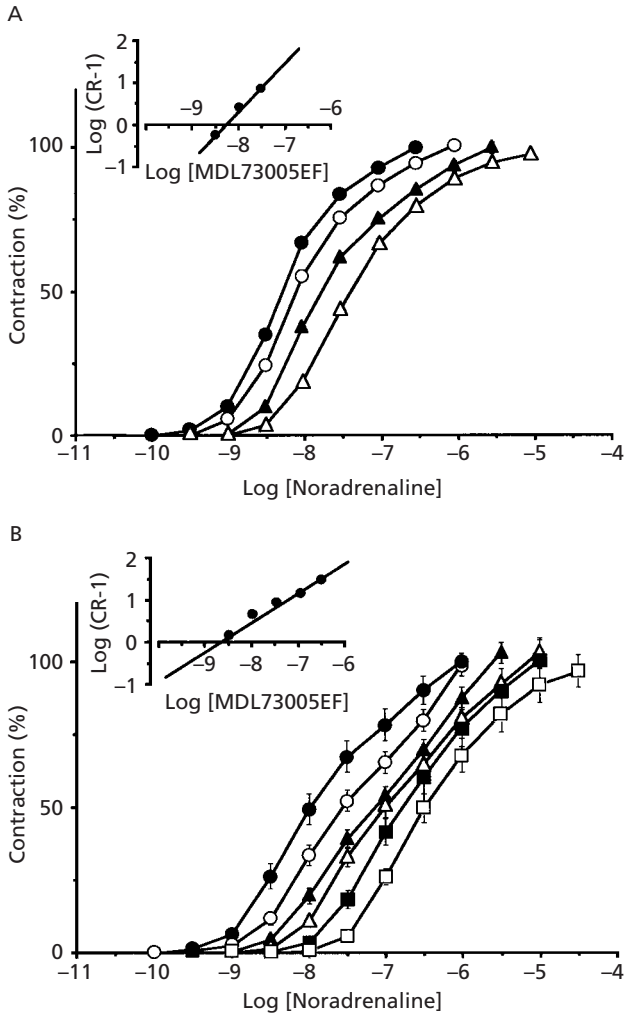

Figure 1 Effect of MDL73005EF on noradrenaline-induced contraction and Schild plots for antagonism between noradrenaline and MDL73005EF in rat thoracic aorta (A) and rabbit iliac artery (B). Ordinate: contraction, expressed as a percentage of the contractile response to noradrenaline (3×10^{-6} M). Abscissa: logarithm of noradrenaline concentration (M). ●, noradrenaline alone; ○, 3×10^{-9} M MDL73005EF; ▲, 10^{-8} M MDL73005EF; △, 3×10^{-8} M MDL73005EF; ■, 10^{-7} M MDL73005EF; □, 3×10^{-7} M MDL73005EF. Inset, Schild plots. Ordinate: logarithm of equi-effective concentration ratio (CR) of noradrenaline minus 1. Abscissa: logarithm of molar concentration of MDL73005EF. Each value is presented as the mean ± s.e.m. (bar) of four separate experiments.

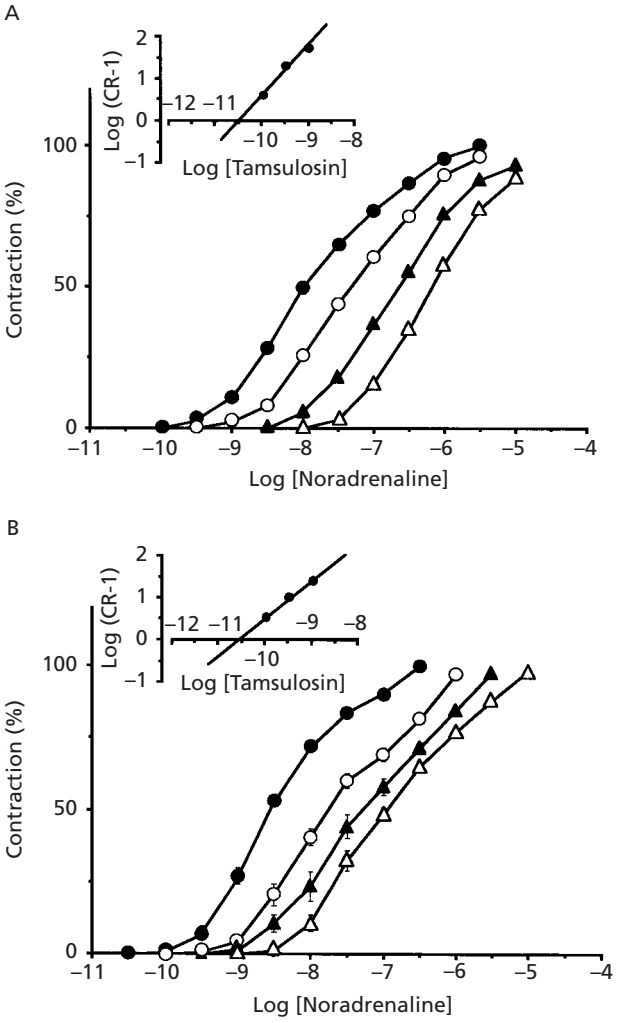

Figure 2 Effect of tamsulosin on noradrenaline-induced contraction and Schild plots for antagonism between noradrenaline and tamsulosin in rat thoracic aorta (A) and rabbit iliac artery (B). Ordinate: contraction, expressed as a percentage of the contractile response to noradrenaline (3×10^{-6} M). Abscissa: logarithm of noradrenaline concentration (M). ●, noradrenaline alone; ○, 10^{-10} M tamsulosin; ▲, 3×10^{-10} M tamsulosin; △, 10^{-9} M tamsulosin. Inset, Schild plots. Ordinate: logarithm of equi-effective concentration ratio (CR) of noradrenaline minus 1. Abscissa: logarithm of molar concentration of tamsulosin. Each value is presented as the mean ± s.e.m. (bar) of four separate experiments.

(n = 4), respectively. Selective α_{1D}-adrenoceptor subtype antagonist MDL73005EF shifted the concentration–response curves for noradrenaline to the right in these vessels (Figure 1). In rat thoracic aorta, the Schild regression obtained from the results of antagonism between MDL73005EF (3–30 nM) and noradrenaline yielded a straight line with a slope of unity (1.08 ± 0.02), suggesting a simple competitive antagonism, and the pA_2 value for MDL73005EF against noradrenaline was 8.30 ± 0.04. In rabbit iliac artery, however, the Schild plot of the results obtained from the inhibition by MDL73005EF (3–300 nM) of noradrenaline yielded a slope significantly different from unity (0.63 ± 0.02), suggesting that noradrenaline acted through at least two receptor populations. Selective α_1-adrenoceptor subtype

Table 1 Effect of MDL73005EF against noradrenaline and slope of Schild plot for antagonism between noradrenaline and MDL73005EF in rat thoracic aorta and rabbit iliac artery.

Arteries	n	pA_2 value	Slope
Rat thoracic aorta	4	8.30 ± 0.04	1.08 ± 0.02
Rabbit iliac artery	4	–	0.63 ± 0.03*

Each value is presented as mean ± s.e.m. of four separate experiments. *$P < 0.05$ compared with unity.

Table 2 Effect of tamsulosin against noradrenaline and slope of Schild plot for antagonism between noradrenaline and tamsulosin in rat thoracic aorta and rabbit iliac artery.

Arteries	n	pA_2 value	Slope
Rat thoracic aorta	4	10.51 ± 0.06	1.02 ± 0.04
Rabbit iliac artery	4	–	0.87 ± 0.03*

Each value is presented as mean ± s.e.m. of four separate experiments. *$P < 0.05$ compared with unity.

antagonist tamsulosin also shifted the concentration–response curves for noradrenaline to the right in rat thoracic aorta and rabbit iliac artery (Figure 2). In rat thoracic aorta, the Schild regression obtained from the results of antagonism between tamsulosin (0.1–1 nM) and noradrenaline yielded a straight line with a slope of unity (1.02 ± 0.04), suggesting a simple competitive antagonism, and the pA_2 value for tamsulosin against noradrenaline was 10.51 ± 0.06. However, in rabbit iliac artery Schild plot of the results obtained from the inhibition by tamsulosin (0.1–1 nM) of noradrenaline yielded a slope significantly different from unity (0.87 ± 0.03), suggesting that noradrenaline acted through at least two receptor populations. These data are summarized in Tables 1 and 2.

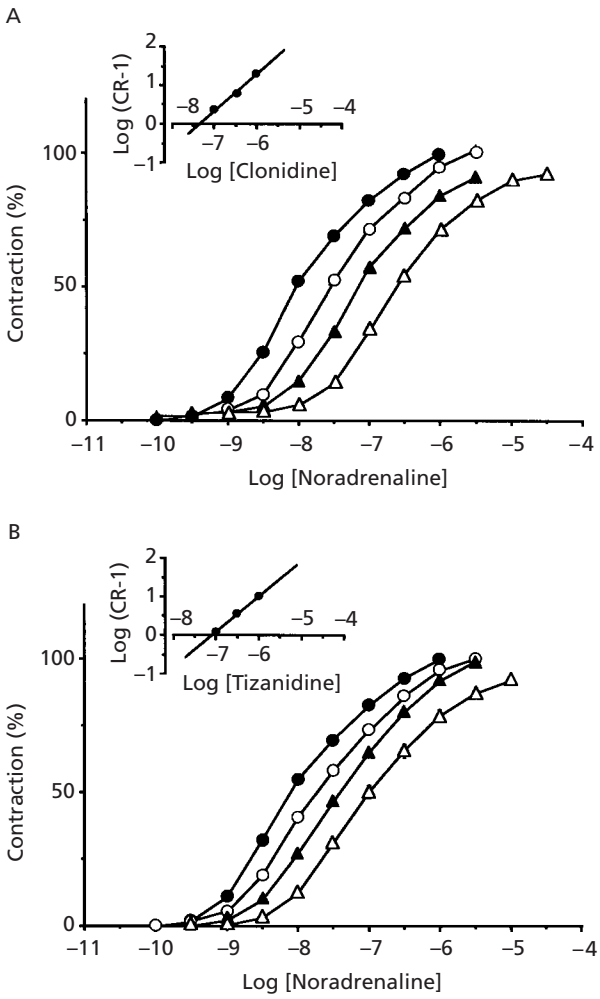

Figure 3 Effect of clonidine and tizanidine on noradrenaline-induced contraction and Schild plot for antagonism between noradrenaline and clonidine (A) and that for antagonism between noradrenaline and tizanidine (B) in rat thoracic aorta. Ordinate: contraction, expressed as a percentage of the contractile response to noradrenaline (3×10^{-6} M). Abscissa: logarithm of noradrenaline concentration (M). ●, noradrenaline alone; ○, 10^{-7} M; ▲, 3×10^{-7} M; △, 10^{-6} M. Inset, Schild plots. Ordinate: logarithm of equi-effective concentration ratio (CR) of noradrenaline minus 1. Abscissa: logarithm of molar concentration of clonidine or tizanidine. Each value is presented as the mean ± s.e.m. (bar) of four separate experiments.

Effects of clonidine and tizanidine

In rat thoracic aorta (Figure 3), clonidine and tizanidine produced a small contraction, and the maximum amplitude of clonidine- and tizanidine-induced contractions was approximately 10% of the noradrenaline-induced contraction. Clonidine and tizanidine shifted the concentration–response curves for noradrenaline to the right (Figure 3). The Schild regression obtained from the results of antagonism between clonidine (100–1000 nM) and noradrenaline yielded a straight line with a slope of unity (1.04 ± 0.03), suggesting a simple competitive antagonism, and the pA_2 value for clonidine against noradrenaline was 7.32 ± 0.04. The Schild regression obtained from the results of antagonism between tizanidine (100–1000 nM) and noradrenaline yielded a straight line with a slope of unity (1.00 ± 0.02), suggesting a simple competitive antagonism, and the

Table 3 pA_2 values for clonidine and tizanidine against noradrenaline and slopes of Schild plot for antagonisms between noradrenaline and these drugs in rat thoracic aorta.

	n	pA_2	Slope
Clonidine	4	7.32 ± 0.04	1.04 ± 0.03
Tizanidine	4	7.08 ± 0.04	1.00 ± 0.02

Each value is presented as mean ± s.e.m. of four separate experiments.

pA_2 value for tizanidine against noradrenaline was 7.08 ± 0.04. These data are summarized in Table 3.

Discussion

MDL73005EF has a higher selectivity for the α_{1D}-adrenoceptor subtype ($pK_i = 8.0 \sim 8.16$) than that for the α_{1A}- ($pK_i = 5.5 \sim 6.1$) or α_{1B}-adrenoceptor subtypes ($pK_i = 6.2 \sim 6.9$) (Saussy et al 1996; Low et al 1998). Tamsulosin also has a higher affinity for α_{1D}- (rat aorta: $pA_2 = 10.1$, $pK_B = 10.1$ and $pK_i = 9.8$) and α_{1A}-adrenoceptor subtypes (rat vas deferens: $pA_2 = 9.52$, $pK_B = 9.7$–10.0 and $pK_i = 9.7$) than it does for the α_{1B}-adrenoceptor subtype (rat spleen: $pA_2 = 8.9$, $pK_B = 8.9$ and $pK_i = 8.9$) (Kenny et al 1996; Noble et al 1997). Partial agonists, such as clonidine and tizanidine, act as competitive antagonists toward the α_{1B}-adrenoceptor subtype (rabbit iris dilator, $pA_2 = 6.05$ and 5.51, respectively), although they act as agonists toward the α_{1A}-adrenoceptor subtype in rabbit thoracic aorta and iliac artery (Takayanagi et al 1991; Satoh et al 1992a, b). The selective properties of α_{1D}-antagonist MDL73005EF demonstrated here support our previous findings in which we reported the co-existence of an α_{1D}-adrenoceptor subtype in addition to the α_{1A}- and α_{1B}-adrenoceptor subtypes in rabbit iliac artery (Satoh et al 1998, 1999), and so we used partial agonists clonidine and tizanidine to characterize the α_{1D}-adrenoceptor subtype in rat thoracic aorta.

As shown in Figures 1A and 2A and Tables 1 and 2, in rat thoracic aorta the concentration–response curves of noradrenaline were potently antagonized by MDL-73005EF and tamsulosin, and the pA_2 values for MDL-73005EF and tamsulosin were 8.30 ± 0.04 and 10.51 ± 0.04, respectively. These values are similar to those for the α_{1D}-adrenoceptor subtype (Saussy et al 1996; Low et al 1998). Figures 1B and 2B show that in rabbit iliac artery the concentration–response curves of noradrenaline were also potently antagonized by MDL73005EF and tamsulosin, and the intercepts of the Schild plots were approximately 8.6 and 10.5, similar to the values ($pK_i = 8.16$ and $pK_B = 10.1$, respectively) for the α_{1D}-adrenoceptor subtype (Saussy et al 1996; Noble et al 1997; Low et al 1998).

This indicates the existence of an MDL73005EF- and tamsulosin-sensitive α_{1D}-adrenoceptor subtype in rabbit iliac artery and also suggests the possibility of a contractile response through this α_{1D}-adrenoceptor subtype for the noradrenaline-induced contractions of smooth muscle in rabbit iliac artery, as reported previously using a selective α_{1D}-adrenoceptor antagonist BMY 7378 (Satoh et al 1998, 1999). These findings suggest that noradrenaline-induced contraction in rat thoracic aorta is predominantly mediated through the α_{1D}-adrenoceptor subtype, that MDL73005EF and tamsulosin have high affinity for the α_{1D}-adrenoceptor subtype, and that an α_{1D}-adrenoceptor subtype contributes to the α_1-adrenoceptor mediation of muscle contraction in rabbit iliac artery. Furthermore, in rat thoracic aorta the slopes of the Schild plots of the results obtained from the inhibition by MDL73005EF and tamsulosin were unity (1.08 ± 0.02 and 1.02 ± 0.04, respectively), indicating that there is a simple competitive antagonist at a single site. However, in rabbit iliac artery the slopes obtained from these antagonists were significantly different from unity (0.63 ± 0.03 and 0.87 ± 0.03, respectively), indicating that there is not a simple competitive antagonism. These findings support the view that in rat thoracic aorta noradrenaline induces contraction through only the α_{1D}-adrenoceptor subtype, but in rabbit iliac artery it induces contraction through not only the α_{1D}-subtype but also others (α_{1A}- and α_{1B}-adrenoceptor subtypes) as we reported previously (Takayanagi et al 1991; Satoh et al 1998, 1999).

In rat thoracic aorta clonidine and tizanidine produced small contractions, the amplitudes of which were approximately 10% of that of noradrenaline-induced contraction, although in rabbit thoracic aorta and iliac artery these two partial agonists produced contractile responses with 50–70% of the maximum amplitude of noradrenaline-induced contraction (Takayanagi et al 1991; Satoh et al 1992b, 1998). As shown in Figure 3, the concentration–response curves of noradrenaline were antagonized by clonidine and tizanidine, and the slopes of Schild plots yielded a straight line with a slope of unity (1.04 ± 0.03 and 1.00 ± 0.02, respectively), and the pA_2 values for clonidine and tizanidine were 7.32 ± 0.04 and 7.08 ± 0.04, respectively. Clonidine and tizanidine have been reported to act as competitive α_{1B}-adrenoceptor antagonists toward noradrenaline in the rabbit iris dilator (Takayanagi et al 1992) and

Takayanagi et al (1991) and Satoh et al (1992b) reported that partial agonists clonidine and tizanidine produced contractions of vessel smooth muscle mediated only through the α_{1A}-adrenoceptor. These findings suggest that partial agonists such as clonidine and tizanidine, which interact with three α_1-adrenoceptor subtypes (α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors), act as agonists toward the α_{1A}-adrenoceptor subtype (rabbit thoracic aorta and iliac artery), and act as antagonists toward the other two subtypes (α_{1B} (rabbit iris dilator) and α_{1D} (rat thoracic aorta)).

In conclusion, this functional study using MDL73005EF and tamsulosin supports the view that in rabbit iliac artery the α_1-adrenoceptors that mediate contractions include pharmacological characteristics of the α_{1D}-adrenoceptor subtype, and that rat thoracic aorta predominantly has a functional α_{1D}-adrenoceptor subtype. Partial agonists clonidine and tizanidine act as competitive antagonists toward the α_{1D}-adrenoceptor subtype.

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