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(±)-Domesticine, a novel and selective α_{1D}-adrenoceptor antagonist in animal tissues and human α_1-adrenoceptors

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Abstract

The pharmacological profile of (±)-domesticine, a novel α_1-adrenoceptor antagonist, was examined in animal tissues and Chinese hamster ovary (CHO) cells expressing cloned human α_1-adrenoceptor subtypes and compared with the properties of BMY-7378 ([8-(2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl)-8-azaspirol [4.5]decane-7,9-dione dihydrochloride], the prototypical α_{1D}-adrenoceptor antagonist. Both (±)-domesticine and BMY-7378 were more potent in inhibiting the phenylephrine-induced contraction in rat thoracic aorta than tail artery or spleen. The selectivity of (±)-domesticine to inhibit phenylephrine-induced contraction in rat thoracic aorta was 32- and 17-fold higher than that in tail artery and spleen, respectively, while that of BMY-7378 it was 125- and 11-fold, respectively. The functional affinity profiles of these compounds for the α_1-adrenoceptor subtypes in animal tissues were consistent with the respective binding affinity profiles in cloned human α_1-adrenoceptor subtypes. (±)-Domesticine displayed a 34- and 9-fold higher selectivity for α_{1d}-adrenoceptor than for α_{1a}- and α_{1b}-adrenoceptor, respectively, while BMY-7378 showed a selectivity for α_{1d}-adrenoceptor of 102-fold higher than that of α_{1a}-adrenoceptor and 21-fold higher than that of α_{1b}-adrenoceptor. Interestingly, in [^{3}H]8-OH-DPAT (8-hidroxy-2-(di-*n*-propyl-amino)tetraline hidrobromide) binding to 5-HT_{1A} receptors of rat cerebral cortex, (±)-domesticine showed a 183-fold higher selectivity for α_{1D}-adrenoceptor relative to 5-HT_{1A} receptor, whereas BMY-7378 displayed a similar affinity at this receptor with respect to the α_{1D}-adrenoceptor (0.89-fold). Both compounds, however, showed a weak affinity for 5-HT_{2A}/5-HT_{2C} receptors in rat frontal cortex. These results suggest that (±)-domesticine is more potent for α_{1D}-adrenoceptor than for α_{1A}- or α_{1B}-adrenoceptor subtypes and it is highly selective compared to 5-HT_{1A} and other receptors.

Keywords: Adrenoceptor subtype; α_{1D}-Adrenoceptor antagonist; (±)-Domesticine; BMY-7378

1. Introduction

Molecular cloning techniques have revealed the existence of at least three α_1-adrenoceptor subtypes: α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor. Pharmacological studies have indicated that these cloned subtypes correspond to native α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes (Bylund et al., 1995; Hieble et al., 1995). The existence of different α_1-adrenoceptors in diverse tissues may provide therapeutic opportunity by selective blocking of the desirable subtypes in target tissue, without affecting other tissues having other subtypes, thus minimizing adverse effects.

Most α_1-adrenoceptor antagonists, including the prototype antagonist prazosin, show little or no selectivity between the three known α_1-adrenoceptor subtypes (Hancock, 1996). The first α_{1A}-adrenoceptor selective antagonist to be identified was WB4101 ((2,6-dimethoxy dimethoxyphenoxyl)aminomethyl-1,4-benzodioxane hydrochloride) (Morrow and Creese, 1986), which has an approximately 20-fold higher affinity for α_{1A}-adrenoceptor than the α_{1B}-adrenoceptor subtype. In addition, the recently identified α_{1A}-adrenoceptor selective antagonists are KMD-3213 ([(−)-(*R*)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy)phenoxyl]ethyl] amino] propyl]indoline-7-carboxamide]) (Shibata et al., 1995), Rec 15/2739 (*N*-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxamide) (Leonardi et al., 1997), JTH-601 ((*N*-(3-

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hydroxy-6-methoxy-2,4,5-trimethylbenzyl)-*N*-methyl-2-(4-hydroxy-2-isopropyl-5-methylphenoxy)ethylamine hemifumarate) (Muramatsu et al., 1996) and others (Hancock, 1996; Patane et al., 1998). Some competitive antagonists including spiperone and cyclazosin have been reported to be moderately selective for α_{1B}-adrenoceptor (Hancock, 1996). Although several α_{1A}-adrenoceptor and α_{1B}-adrenoceptor selective antagonists are available, an ideal selective ligand that recognizes the α_{1D}-adrenoceptor subtype among multiple receptor subtypes is not available (Zhong and Minneman, 1999). For example, BMY-7378 displays selective antagonistic activity for the α_{1D}-adrenoceptor subtype relative to α_{1A}- and α_{1B}-adrenoceptor (Goetz et al., 1995), but it shows a more potent antagonistic action at 5-HT_{1A} receptors compared to α_{1D}-adrenoceptor (Minarini et al., 1998).

A group of aporphine alkaloids has been shown to have α_1-adrenoceptor antagonistic properties in vascular smooth muscle (Ivorra et al., 1992; Ivorra et al., 1993; Ko et al., 1993; Ko et al., 1994; Chulia et al., 1994; Chulia et al., 1996; Martinez et al., 1999). In the course of our survey of pharmacologically active substances, we have focused our attention on a potent and selective blocker for the α_{1D}-adrenoceptor subtype, which does not affect other receptors. Recently, we found that (±)-domesticine (Fig. 1), an aporphine alkaloid, inhibited the contraction of rat thoracic aorta induced by phenylephrine (observation data). The detailed pharmacological properties of this compound have not been reported yet. In the present study, we examined the potency and selectivity of (±)-domesticine on α_1-adrenoceptor subtypes using animal tissues, and Chinese hamster ovary (CHO) cells expressing cloned human α_1-adrenoceptor subtypes and compared them with the findings for BMY-7378. This is the first report showing that (±)-domesticine is a potent α_{1D}-adrenoceptor antagonist and is highly selective compared to 5-HT_{1A} and other receptors.

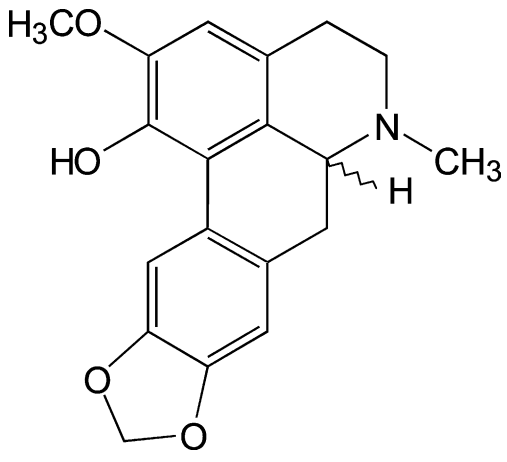

Fig. 1. Chemical structure of (±)-domesticine.

2. Materials and methods

2.1. Peripheral tissue assays

All of the animals used in this study were treated according to the principles and guidelines on animal care of Tohoku University. Male Wistar rats (220–250 g) or male albino rabbits (2–3 kg) were killed by cervical dislocation. Rabbit/rat thoracic aorta and rat tail artery were dissected and connective tissues were carefully removed. Helical strips of the thoracic aorta (approximately 1.5–2 mm in width and 10 mm in length) and tail artery (approximately 3–5 mm in length) were denuded of endothelium by gently rubbing the endothelial surface with cotton pellets. The lack of endothelium was checked by the abolition of carbachol (1 μM)-induced relaxation. The rat spleen was also removed and bisected longitudinally into two strips. The strip was suspended in an organ bath containing 6 ml of Krebs–Henseleit buffer (KH) (supplemented with 1 μM propanolol and 0.1 μM yohimbine when phenylephrine was used as an agonist) at 37 °C and aerated with 95% O_2–5% CO_2. The composition of the KH was 119 mM NaCl, 5.4 mM KCl, 25.2 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4 \cdot 7H_2O$, 2.5 mM $CaCl_2 \cdot 2H_2O$ and 11 mM glucose. An initial load of 1 g was applied to each preparation and maintained throughout the 75- to 90-min equilibration period. The tissues were contracted with agonist (phenylephrine /5-HT, 10 μM in thoracic aorta; phenylephrine, 100 μM in tail artery and spleen; histamine, 100 μM in rabbit thoracic aorta) and washed for a 30-min period (the repeat phenylephrine curve for the spleen was begun 2 h after the first curve). Isometric contractions were obtained in response to cumulative additions of agonist in the absence and presence of antagonist (incubated for 30 min). Dose-dependent contraction curves for the agonist (phenylephrine 5-HT, 0.003–100 μM in thoracic aorta; phenylephrine, 0.03–300 μM in tail artery; spleen; histamine, 0.03–300 μM in rabbit thoracic aorta) in the presence of various concentrations of antagonist were normalized with the control dose–response curve, of which the maximum response was taken as 100%.

Contractile studies for guinea pig left atria were prepared as described previously (Kobayashi et al., 1991). Left atria were excised from male guinea pig (250–350 g) and mounted vertically in a 20-ml organ bath filled with a modified Krebs–Ringer bicarbonate solution. The solution was aerated with 95% O_2–5% CO_2 and temperature was maintained at 30 °C. One end of the tissue was connected to a force-displacement transducer and the other end was secured to a plastic tissue holder containing platinum stimulating electrodes. The left atria preparations were electrically stimulated by square-wave pulses (2 Hz, 5 V). Resting tension of 1 g was applied to the atria. The tissues were equilibrated for at least 1 h before starting the experiments. Contractile response was expressed as a percentage of the maximal response (100%) to isoproterenol (30 nM).

2.2. Cloning of the human α_1-adrenoceptor subtypes

The human α_{1a}- and α_{1b}-adrenoceptor cDNA clones were isolated from human prostate and aorta cDNA libraries, respectively. The cDNA for human α_{1d}-adrenoceptor was kindly provided by Dr. Minneman. Each clone was constructed into a mammalian expression vector pCR3 (Invitrogen) and transfected into Chinese hamster ovary (CHO) cells with Lipofectamin (GIBCO). Stable transformant was screened and selected in media containing 500 mg/ml G418 (GIBCO). The receptor density expressed in CHO cells was 3500, 1300 and 130 fmol/mg protein for the α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes, respectively.

2.3. Radioligand binding assays in CHO cells expressing human cloned α_1-adrenoceptors

The harvested CHO cells expressing human cloned α_1-adrenoceptor subtypes were suspended in ice-cold assay buffer (50 mM Tris–HCl, 2 mM EDTA, at pH 7.4), sonicated and centrifuged at $3000 \times g$ for 5 min. The supernatant was then centrifuged at $80{,}000 \times g$ for 30 min and the pellet was resuspended in assay buffer for the binding experiment. All procedures were performed at 4 °C (Takahashi et al., 2000). In saturation binding experiments, the membranes were incubated with 10–3000 pM of [^{3}H]prazosin for 45 min at 30 °C. In competition binding experiments, the membranes were incubated at 30 °C for 45 min with 200 pM [^{3}H]prazosin and different concentrations of unlabelled drugs. The binding interaction was terminated by filtration through a glass fiber filter (Whatman GF/C) pretreated with 0.3% (w/v) polyethyleneimine under vacuum, and the residue was washed with three applications of 4 ml of 50 mM Tris–HCl, pH 7.4. The radioactivity retained on the filters was determined by liquid scintillometry in 5 ml of tritosol scintillation cocktail. The total binding was defined as that measured in the absence of a competing agent and at least 10 concentrations of compounds, ranging from 10^{-4} to 3×10^{-11} M, were used to inhibit the binding of [^{3}H]prazosin. Nonspecific binding was determined in the presence of 10 μM phentolamine and was about 10% of the total binding. All assays were carried out at least four times in duplicate. Protein concentration was quantified by the method of Bradford (1976) using bovine serum albumin as the standard.

2.4. Binding assays at the 5-HT$_{1A}$ and 5-HT$_{2A/2C}$ receptors in rat brain membrane

Radioligand binding assays were performed as previously reported by Herndon et al. (1992). Briefly, cerebral (5-HT$_{1A}$ receptor) or frontal (5-HT$_{2A/2C}$ receptors) cortical regions of male Wistar rats (220–250 g) were dissected on ice and homogenized (1:10 w/v) in ice-cold buffer solution (50 mM Tris–HCl, 0.5 mM EDTA and 10 mM $MgCl_2$ at pH 7.4). The homogenate was centrifuged at $50{,}000 \times g$ for 15 min at 4 °C and the supernatant was discarded. The final pellet was resuspended in 5 volumes of 50 mM Tris–HCl buffer (containing 0.3 M sucrose), distributed in 1 ml aliquots in plastic vials (Nunc) at a final concentration of 10 mg/ml and frozen at −80 °C until required. Assays were performed in 1 ml volume containing 350 μg membrane proteins with 1 nM [^{3}H]8-OH-DPAT for the 5-HT$_{1A}$ receptor, 0.75 nM [^{3}H]ketanserin for the 5-HT$_{2A}$ receptor and 2 nM [^{3}H]mesulergine for the 5-HT$_{2C}$ receptor. Tubes were incubated for 30 min (5-HT$_{1A}$ receptor) or 20 min (5-HT$_{2A/2C}$ receptors) at 37 °C and filtered on Whatman GF/C glass fiber filters (presoaked in 0.3% polyethyleneimine) under vacuum. The filters were washed with 5 ml of 50 mM Tris–HCl buffer. The total binding was defined as that measured in the absence of a competing agent. 8-OH-DPAT (1 μM), ketanserin (10 μM) and mianserine (1.0 μM) were used to define nonspecific binding in the 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, respectively, and represented about 10% to 15% of the total binding. Moreover, at least 10 concentrations of compounds, ranging from 10^{-4} to 3×10^{-11} M, were used to inhibit the binding of [^{3}H]ligands. All assays were carried out at least four times in duplicate. Protein concentration was quantified by the method of Bradford (1976) using bovine serum albumin as the standard.

2.5. Data analysis

Concentration–effect curves were plotted by using nonlinear curve-fitting methodologies to a form of the logistic equation for estimation of mid-point parameter. Schild plots were constructed to estimate the pA_2 value of the antagonist and the slope of regression line from each experimental series, which generally comprised at least three different concentrations (Arunlakshana and Schild, 1959). The dissociation constant (K_B) of each antagonist was determined from the following equation: K_B = antagonist [M]/(CR − 1), where CR is the concentration ratio between the EC_{50} values of control doses and those of the curves for antagonist treatment. Antagonist affinity estimates (as pA_2) were obtained by the construction of Schild plot regressions and constructed by plotting the log (CR-1) against the − log of antagonist concentration. If the regression was linear and had a slope not significantly different from unity ($P > 0.05$), the regression was recalculated with a constrained slope of unity.

For binding experiment, the analysis of radioligand binding data was performed with LIGAND (Munson and Rodbard, 1980), a nonlinear curve-fitting program. Specific [^{3}H]ligand binding was determined experimentally from the difference between counts in the absence and presence of nonspecific binding. The ability of drugs to inhibit specific [^{3}H]ligand binding was estimated from the IC_{50} values, which are the molar concentration of unlabelled drug necessary to displace 50% of the specific binding of [^{3}H]ligand. The IC_{50} values and Hill slope were calculated from the equation $Y = A/(X/X_o)^p$, where $A = B_{max}$, $X_o = IC_{50}$ and p = Hill slope (Kenakin, 1982, 1997). The inhibition

Table 1
Antagonistic activity of (±)-domesticine and BMY-7378

Tissues/ receptor	(±)-Domesticine		BMY 7378	
	pA_2	Slope	pA_2	Slope
Rat aorta α_{1D}	7.931 ± 0.030	0.931 ± 0.078	8.516 ± 0.028	0.949 ± 0.081
Rat spleen α_{1B}	6.690 ± 0.021	0.997 ± 0.051	7.487 ± 0.016	1.033 ± 0.045
Rat tail artery α_{1A}	6.421 ± 0.042	1.141 ± 0.039	6.416 ± 0.041	0.911 ± 0.114
Rat aorta 5-HT_{2A}	6.071 ± 0.009	0.976 ± 0.024	6.328 ± 0.004	0.998 ± 0.009

Phenylephrine or 5-HT concentration–response curve was obtained in the absence and presence of antagonist, pA_2 (means ± S.E.M., at least five times in duplicate).

constant, K_i, was calculated from the equation $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration of [^{3}H]ligand and K_d is the equilibrium dissociation constant (Cheng and Prusoff, 1973). The values are presented as the negative logarithm of the pK_i. Data are shown as means ± S.E.M. Statistical significance was calculated with analysis of variance (ANOVA). A P value of less than 0.05 was considered to indicate a significant difference.

2.6. Drugs and chemicals

(±)-Domesticine (Fig. 1) was synthesized according to a procedure previously described in the literature (Hoshino et al., 1975). [^{3}H]prazosin (specific activity, 11.2 Ci/mmol), [^{3}H]ketanserin (specific activity, 63.8 Ci/mmol), [^{3}H]mesulergine (specific activity, 86 Ci/mmol), and [^{3}H]8-OH-DPAT (specific activity, 234 Ci/mmol) were obtained from DuPont/NEN Research Products (Wilmington, MA, USA). BMY-7378 ([8-(2-[4-(2-methoxy-phenyl)-1-pierazinyl]ethyl)-8-azaspirol [4.5]decane-7,9-dione dihydrochloride] was purchased from TOCRIS (UK). 5-HT creatine sulfate, phenylephrine hydrochloride, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propyl-amino)tetraline hidrobromide), propranolol

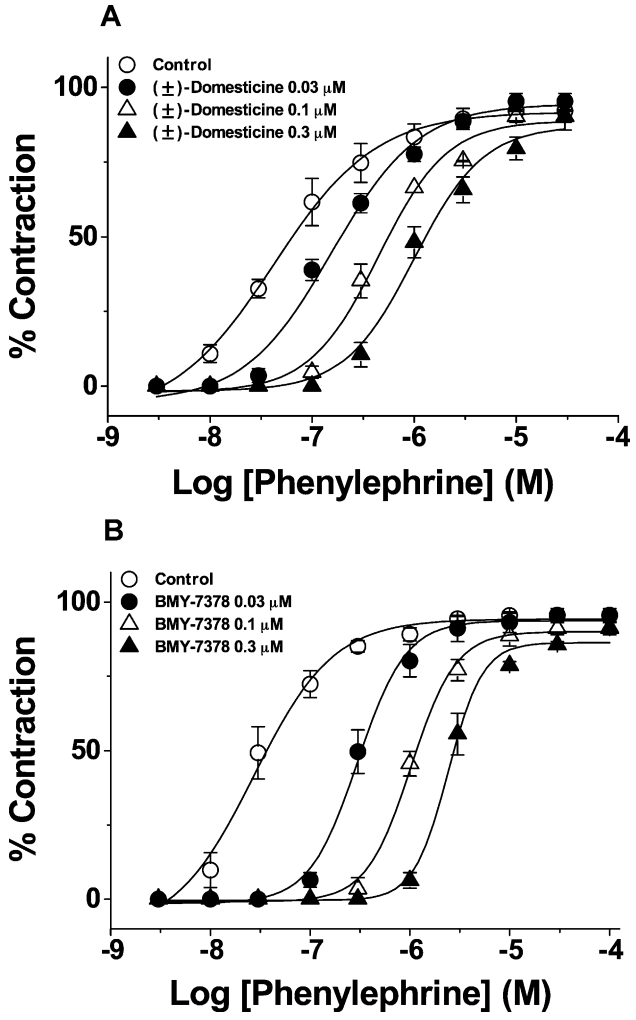

Fig. 2. Effects of (±)-domesticine (A) and BMY-7378 (B) on phenylephrine-induced contraction in the rat thoracic aorta. Each point is the mean of at least six times in duplicate and the vertical lines show S.E.M.

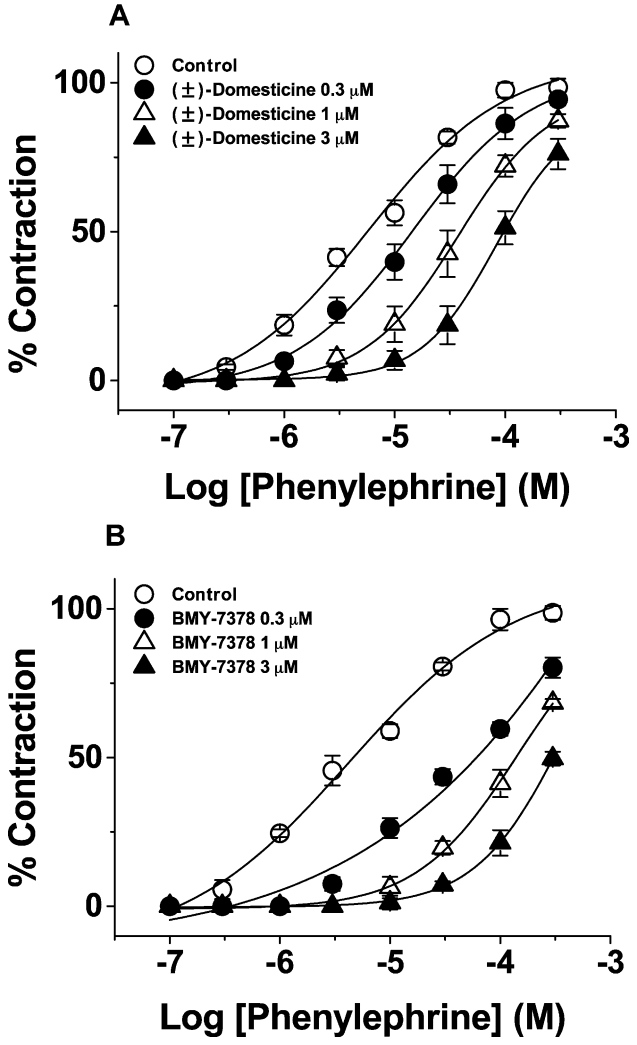

Fig. 3. Effects of (±)-domesticine (A) and BMY-7378 (B) on phenylephrine-induced contraction in the rat spleen. Each point is the mean of at least five times in duplicate and the vertical lines show S.E.M.

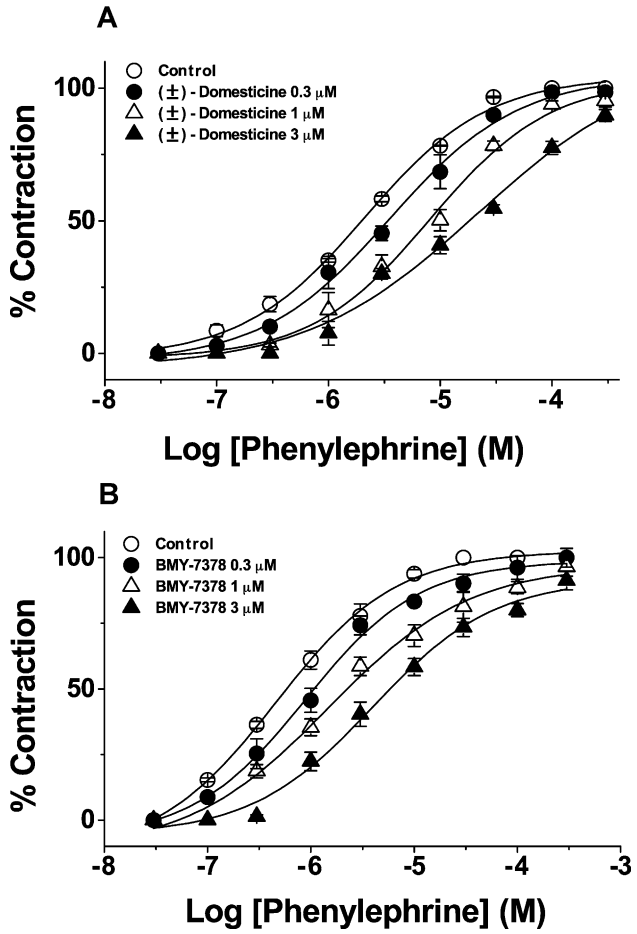

Fig. 4. Effects of (±)-domesticine (A) and BMY-7378 (B) on phenylephrine-induced contraction in the rat tail artery. Each point is the mean of at least five times in duplicate and the vertical lines show S.E.M.

hydrochloride, prazosin hydrochloride, phentolamine hydrochloride and yohimbine hydrochloride were from Sigma (St Louis, MO, USA). All other chemicals used in this study were of analytical grade and obtained from commercial sources. (±)-Domesticine was dissolved in dimethylsulphoxide (DMSO) and diluted in assay buffer solution (50 mM Tris–HCl, at pH 7.4) for binding studies or KH buffer for the functional experiment. Other drugs were dissolved in distilled water.

3. Results

3.1. Contractile studies

The effects of the tested compounds in peripheral tissue are shown in Table 1. (±)-Domesticine as well as BMY-7378 inhibited the concentration–response curve for phenylephrine in rat thoracic aorta, spleen and tail artery, indicating that their actions at the receptors are those of antagonists. (±)-Domesticine and BMY-7378 displayed a higher potency to inhibit the concentration–response curve for phenylephrine in rat thoracic aorta (Fig. 2; Table 1). In rat spleen, these compounds showed a lower potency than in rat thoracic aorta (Fig. 3; Table 1). The Schild plot of the result obtained from the inhibition by both compounds in thoracic aorta and spleen yielded a straight line with a slope of unity (Table 1). In rat tail artery, (±)-domesticine and BMY-7378 exhibited a parallel rightward shift of the concentration–response curve for phenylephrine (Fig. 4); however, the Schild plot from the inhibition by both compounds showed a slope significantly different from unity (Table 1). The selectivity of (±)-domesticine to inhibit phenylephrine-induced contraction in rat thoracic aorta was 32- and 17-fold higher than that in tail artery and spleen, respectively, while that of BMY-7378 it was 125- and 11-fold, respectively (Table 3). The concentration–response curve for 5-HT of both compounds in rat thoracic aorta was slightly shifted to the right in a parallel manner (Table 1). In rat thoracic aorta, (±)-domesticine and BMY-7378 were 72- and 154-fold more selective than 5-HT for inhibiting the concentration–response curve of phenylephrine, respectively (Table 3). Moreover, the concentration–contractile response curves for histamine (0.03–300 μM) in rabbit thoracic aorta or KCl (0.1–60 mM) in rat thoracic aorta or inotropic responses to isoproterenol (30 nM) in guinea pig left atria were not affected by (±)-domesticine at concentrations of up to 10 μM (data not shown).

3.2. Binding studies with the rat brain membrane

The binding affinities of the tested compounds for the rat brain membrane are shown in Table 2. (±)-Domesticine had less affinity to inhibit the [^{3}H]8-OH-DPAT binding to the 5-HT_{1A} receptors in rat cerebral cortex (Fig. 5; Table 2). In

Table 2
Radioligand binding affinities for (±)-domesticine and BMY-7378

Tissue/ receptor	(±)-Domesticine		BMY-7378	
	pK_i	Hill slope	pK_i	Hill slope
Clone human α_{1d}	7.903 ± 0.063	0.975 ± 0.081	8.671 ± 0.064	1.002 ± 0.054
Clone human α_{1b}	6.961 ± 0.043	0.997 ± 0.047	7.333 ± 0.057	1.020 ± 0.060
Clone human α_{1a}	6.373 ± 0.031	0.986 ± 0.087	6.661 ± 0.071	1.071 ± 0.051
Rat cerebral cortex 5-HT_{1A}	5.641 ± 0.120	0.942 ± 0.098	8.723 ± 0.071	1.000 ± 0.109
Rat frontal cortex 5-HT_{2A}	5.950 ± 0.019	0.972 ± 0.090	6.230 ± 0.036	1.071 ± 0.030
Rat frontal cortex 5-HT_{2C}	5.461 ± 0.081	0.931 ± 0.030	6.650 ± 0.048	0.960 ± 0.080

Data expressed as radioligand binding, pK_i (means ± S.E.M., at least four times in duplicate).

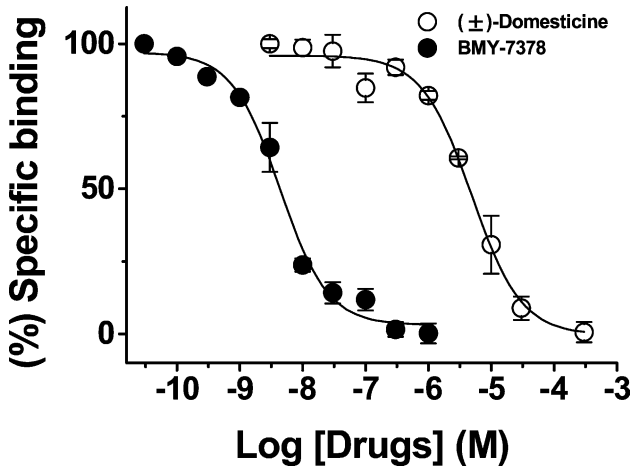

Fig. 5. Concentration–inhibition curve for (±)-domesticine (A) and BMY-7378 (B) in [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptor. Membranes from rat cerebral cortex were incubated with [^{3}H]8-OH-DPAT (1 nM) and varying concentrations of (±)-domesticine and BMY-7378. Each point is the mean of at least four times in duplicate and the vertical lines show S.E.M.

contrast, BMY-7378 had a high affinity for this receptor (Fig. 5; Table 2). (±)-Domesticine was 183-fold more selective for inhibiting the concentration–response curve of phenylephrine in thoracic aorta than for inhibiting the [^{3}H]8-OH-DPAT binding to the 5-HT$_{1A}$ receptors in rat cerebral cortex (Table 3). BMY-7378 showed a similar affinity for this receptor with respect to the result from rat thoracic aorta (0.83-fold). These compounds only slightly inhibited the [^{3}H]ketanserin and [^{3}H]mesulergine binding to the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in rat frontal cortex (Table 2). (±)-Domesticine was 95- and 295-fold more selective for inhibiting the concentration–response curve of phenylephrine in thoracic aorta than the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, respectively, while BMY-7378 was 193- and 73-fold more selective (Table 3). The Hill slope of the result obtained from all experiments yielded not significantly different from 1.0 ($P<0.05$), suggesting that (±)-domesticine and BMY-7378 interact with one site for [^{3}H]ligands (Table 2).

Table 3
Receptor subtypes selectivity of (±)-domesticine and BMY-7378

Tissue/cell	Receptor subtype selectivity	Compound	
		(±)-Domesticine	BMY-7378
Animal tissues	α_{1A}/α_{1D}	32	125
	α_{1B}/α_{1D}	17	11
	5-HT$_{1A}/\alpha_{1D}$	183	0.83
	5-HT$_{2A}/\alpha_{1D}$[a]	72	154
	5-HT$_{2A}/\alpha_{1D}$[b]	95	193
	5-HT$_{2C}/\alpha_{1D}$	295	73
Human α_1ARs	α_{1a}/α_{1d}	34	102
	α_{1b}/α_{1d}	9	21

[a] 5-HT$_{2A}$ (rat aorta).
[b] 5-HT$_{2A}$ (rat brain).

3.3. Binding studies with the cloned human α_1-adrenoceptor subtypes

Membrane preparations from CHO cells stably expressing the human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor showed saturable binding of [^{3}H]prazosin with K_d values of 223.4, 45.0 and 93.7 pM, respectively (data not shown). The binding affinities of the tested compounds for the cloned human α_1-adrenoceptor subtypes are shown in Table 2. Both (±)-domesticine and BMY-7378 inhibited [^{3}H]prazosin binding to human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes in a concentration-dependent manner (Fig. 6). The Hill slope of the result obtained from the competition by both compounds yielded not significantly different from 1.0 ($P<0.05$), suggesting that (±)-domesticine and BMY-7378 interact with one site for [^{3}H]prazosin (Fig. 6; Table 2). The binding affinities of both compounds for the α_{1a}-

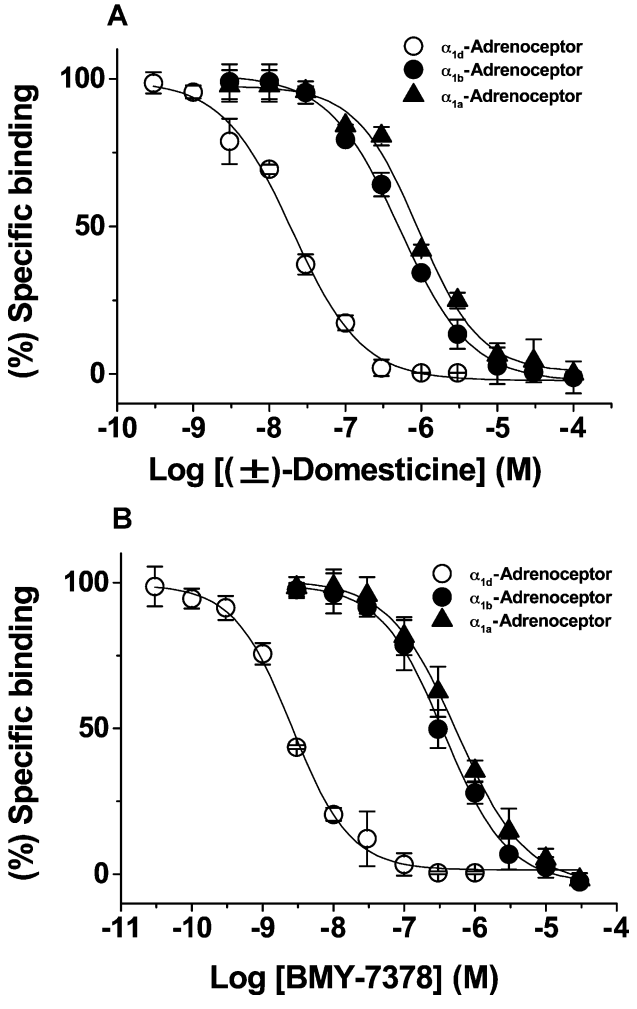

Fig. 6. Concentration–inhibition curve for (±)-domesticine (A) and BMY-7378 (B) in [^{3}H]prazosin binding to α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptor. Membranes from CHO cells transfected with human α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptor were incubated with [^{3}H]prazosin (0.2 nM) and varying concentrations of (±)-domesticine and BMY-7378. Each point is the mean of at least four times in duplicate and the vertical lines show S.E.M.

and α_{1b}-adrenoceptor subtypes were analyzed to assess the degree of receptor subtype selectivity. These compounds showed a low affinity for α_{1a}- and α_{1b}-adrenoceptor subtypes (Table 2). (±)-Domesticine displayed a higher degree of selectivity for α_{1d}-adrenoceptor subtype than for α_{1a}- and α_{1b}-adrenoceptor with ratios of 34- and 9-fold, respectively, while BMY-7378 showed ratios of 102- and 21-fold, respectively (Table 3).

4. Discussion

We characterized (±)-domesticine as a novel type of α_{1D}-adrenoceptor antagonist using animal tissues and CHO cells expressing cloned human α_1-adrenoceptor subtypes. In the present work, the tension experiment using rat thoracic aorta, tail artery and spleen showed that (±)-domesticine inhibited the concentration–response curve of phenylephrine in rat thoracic aorta by 32- and 17-fold higher than that in tail artery and spleen, respectively, while that by BMY-7378, a prototype α_{1D}-adrenoceptor antagonist, it was 125- and 11-fold, respectively. It is known that the α_{1D}-adrenoceptor subtype exists primarily in rat thoracic aorta (Hussain and Marshall, 1997, 2000; Kenny et al., 1995), whereas the α_{1B}-adrenoceptor subtype is predominant in the spleen (Burt et al., 1995). Consistent with those reports, our results showed that the Schild regression obtained from the inhibition by both (±)-domesticine and BMY-7378 yielded a straight line with a slope of unity, suggesting that they are simple competitive antagonists. Therefore, the effect of these compounds to inhibit phenylephrine-induced contraction in rat thoracic aorta and spleen could be α_{1D}-adrenoceptor and α_{1B}-adrenoceptor subtypes, respectively. In rat tail artery, however, the Schild plot from the inhibition by both compounds showed a slope significantly different from unity, indicating that phenylephrine acts via more than one receptor population. This finding was consistent with the reports stating that in rat tail artery at least two α_1-adrenoceptor subtypes are expressed, one of which displays the pharmacology of the α_{1A}-adrenoceptor, while the other remains to be defined (Lachnit et al., 1997; Mita and Walsh, 1997). On the other hand, both compounds showed only slight inhibition of 5-HT-induced contraction in rat thoracic aorta (5-HT_{2A}). The potency of (±)-domesticine and BMY-7378 for blocking the 5-HT_{2A} receptor was 72- and 154-fold less than that to block the α_{1D}-adrenoceptor in rat thoracic aorta. In addition, (±)-domesticine had no effect on the contractile response of thoracic aorta to histamine or KCl and on the positive inotropic response of left atria to isoprotenerol (unpublished data). These results suggest that (±)-domesticine is a selective α_{1D}-adrenoceptor antagonist and possesses a weak 5-HT_{2A} receptor blocking activity without affecting H_1-receptor, β_1-adrenoceptor as well as voltage-dependent calcium channels in animal tissues.

The α_1-adrenoceptor family comprises at least three cloned subtypes, the α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes (Bylund et al., 1995; Hieble et al., 1995). These subtypes have distinctive pharmacological characteristics with a subtype selective ligand (Graham et al., 1996). It is well known that the classical α_1-adrenoceptor antagonist prazosin is a potent but not selective ligand of the three human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes (Shibata et al., 1995; Kenny et al., 1995; Takahashi et al., 2000). The selectivity of (±)-domesticine and BMY-7378 for the α_{1d}-adrenoceptor was further demonstrated in binding studies using cloned human α_1-adrenoceptor subtypes. (±)-Domesticine and BMY-7378 inhibited [^{3}H]prazosin binding to clone α_1-adrenoceptor subtypes. (±)-Domesticine had a selectivity for α_{1d}-adrenoceptor that is 34- and 9-fold higher than that for α_{1a}- and α_{1b}-adrenoceptor, respectively, while for BMY-7378 it was 102- and 21-fold, respectively. These results indicate that (±)-domesticine as well as BMY-7378 shows selectivity for α_{1D}-adrenoceptor not only in animal tissues but also in cloned human recombinant α_1-adrenoceptor subtypes.

A major factor that has limited the ability to conduct definitive in vivo studies on the role of the α_{1D}-adrenoceptor has been the absence of highly potent and selective ligands devoid of ancillary pharmacology (Carroll et al., 2001). An interesting finding from the present study was that (±)-domesticine displayed a 183-fold selectivity for α_{1D}-adrenoceptor relative to the 5-HT_{1A} receptor. In contrast, BMY-7378 only showed a 0.89-fold selectivity for the α_{1D}-adrenoceptor compared to 5-HT_{1A} receptors, indicating that BMY-7378 was equally potent for α_{1D}-adrenoceptor and 5-HT_{1A} receptors. In addition, both compounds were found to have considerably less affinity for the 5-HT_{2A} and 5-HT_{2C} receptors in rat brain frontal cortex compared to α_{1D}-adrenoceptor.

Small structural changes of aporphine alkaloids may lead to dramatic changes in their pharmacological profiles (Hedberg et al, 1995). For example, (−)11-hydroxy-10-methylaporphine is a potent agonist for the 5-HT_{1A} receptor, whereas (+)11-hydroxy-10-methylaporphine is a potent 5-HT_{1A} receptor antagonist. Interestingly, (±)11-hydroxy-10-methylaporphine demonstrated almost no pharmacological activity for the 5-HT_{1A} receptor (Cannon et al, 1993). Consistent with this study, the lack of activity of (±)-domesticine for the 5-HT_{1A} receptor could be due to the effect of the racemic mixture of this compound and because the two enantiomers nullify each other's effects. However, the activity of each enantiomer of (±)-domesticine on the α_1-adrenoceptor subtypes could not be precluded in the present study.

In conclusion, (±)-domesticine is a selective and potent α_{1D}-adrenoceptor antagonist in both the animal tissues and cloned human α_1-adrenoceptor subtypes. Although this compound is not as potent as the prototypical α_{1D}-adrenoceptor antagonist BMY-7378, (±)-domesticine has a low affinity for the 5-HT_{1A} receptors. This is in contrast to BMY-7378, which has a high affinity for this receptor. This gives (±)-domesticine an advantage over BMY-7378 for

evaluating the in vivo functional role of α_{1D}-adrenoceptor and may be promising for the development of new drugs.

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