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Characterization of α_{1D}-adrenoceptor subtype in rat myocardium, aorta and other tissues

Xing Fei Deng,* Sylvain Chemtob & [1]Daya R. Varma

Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6 and *Department of Pediatrics and Pharmacology, Ste-Justine Hospital, Montreal, Quebec, Canada H3T 1C5

1 This study was done to characterize the functional role of α_{1D}-adrenoceptors in rat myocardium, aorta, spleen, vas deferens and prostate by use of the selective antagonist BMY 7378.

2 BMY 7378 inhibited [^{3}H]-prazosin binding to aortic membranes with a potency (pK_i 9.8±0.40) approximately 100 fold higher than in right ventricular membranes (pK_i 7.47±0.11) and approximately 1,000 fold higher than that in plasma membranes of the prostate (pK_i 6.62±0.39), vas deferens (pK_i 6.67±0.15), salivary gland (pK_i 6.46±0.38) and liver (6.58±0.06).

3 BMY 7378 antagonized the positive inotropic effects of phenylephrine (in the presence of 1 μM propranolol) on right ventricles (pA_2 7.0±0.11), left atria (pK_B 7.04±0.18) and papillary muscles (pK_B 6.9±0.1) and inhibited phenylephrine-induced increase in inositol phosphates.

4 BMY 7378 was approximately 100 fold more potent as an antagonist of phenylephrine on aortic strips (pA_2 9.0±0.13) than on vas deferens (pK_B 7.17±0.08) and spleen (pK_B 7.16±0.21); it was ineffective on the prostate.

5 Chloroethylclonidine suppressed the maximal effects of phenylephrine on spleen; 5-methylurapidil antagonized the effects of phenylephrine on aortic strips (pA_2 7.98±0.08), vas deferens (pK_B 8.89±0.07) and prostate (pK_B 8.85±0.21).

6 BMY 7378 caused a dose (0.1-100 nmol kg^{-1})-dependent decrease in mean blood pressure of urethane-anaesthetized rats and its hypotensive efficacy was equal to that of hexamethonium.

7 The data suggest that α_{1D}-adrenoceptors play a significant role in rat aorta, a minor role in the heart, vas deferens and spleen and virtually no role in the prostate.

Keywords: BMY 7378; α_{1D}-adrenoceptors; myocardium; prostate; salivary glands; aorta; vas deferens; inositol phosphates

Introduction

Three subtypes of α_1-adrenoceptors have been identified; these are currently designated as α_{1A}, α_{1B} and α_{1D} and correspond to cloned subtypes previously named as α_{1c}, α_{1b} and $\alpha_{1a/d}$, respectively (Hieble *et al.*, 1995). The predominant α_{1A}-adrenoceptors in the salivary gland and prostate are of the α_{1A} subtype (Michel *et al.*, 1989; Faure *et al.*, 1994; Forray *et al.*, 1994; Scofield *et al.*, 1995). The rat liver mainly contains the α_{1B} subtype (Han *et al.*, 1987; Faure *et al.*, 1994). The expression of α_{1D}-adrenoceptor mRNA is high in vas deferens, cerebral cortex, aorta and adrenals, intermediate in the spleen, trachea and testis, relatively low in the heart and kidney and undetectable in the prostate, liver and salivary glands (Faure *et al.*, 1994; Price *et al.*, 1994; Rokosh *et al.*, 1994; Scofield *et al.*, 1995).

α_{1A}-Adrenoceptors exhibit high affinity for WB4101, (+)-niguldipine and 5-methylurapidil (5-MU) and α_{1B}-adrenoceptors are inactivated by the alkylating agent chloroethylclonidine (CEC) (Morrow & Creese, 1986; Minneman *et al.*, 1988); these antagonists have been extensively used for the pharmacological characterization of α_{1A}- and α_{1B}-adrenoceptors (Terzic *et al.*, 1993). By comparison, the pharmacological characterization of α_{1D}-adrenoceptors has lagged behind. The 5- hydroxytryptamine $(5\text{-HT})_{1A}$ antagonist/partial agonist BMY 7378 (Yocca *et al.*, 1987) has recently been shown to be a highly selective α_{1D}-adrenoceptor antagonist (Goetz *et al.*, 1995; Kenny *et al.*, 1995; Piascik *et al.*, 1995; Testa *et al.*, 1995). In the present study, we used BMY 7378 to characterize functions of α_{1D}-adrenoceptors in the rat heart, aorta, vas deferens, spleen and prostate. In order to assess the antagonistic selectivity of BMY 7378 for the α_{1D} subtype, binding studies were also done with salivary glands, which primarily express α_{1A}-adrenoceptors (Michel *et al.*, 1989; Faure *et al.*, 1994; Price *et al.*, 1994; Rokosh *et al.*, 1994; Scofield *et al.*, 1995) and the liver, which mainly contain α_{1B}-adrenoceptors (Han *et al.*, 1987; Faure *et al.*, 1994).

Methods

Animals

Adult (250-400 g) male Sprague-Dawley rats (Charles River, St-Constant, Quebec, Canada) were used according to a protocol of the McGill University Animal Care Committee. Animals were maintained on a 12 h light (07 h 00 min-19 h 00 min) and 12 h dark schedule at 22-25°C and 50-70% humidity and fed *ad libitum* rat chow and tap water. Rats were decapitated; the heart, thoracic aorta, submaxillary salivary glands, prostate, spleen and the liver were quickly removed for different *in vitro* studies. For blood pressure measurements, rats were anaesthetized with 1 g kg^{-1} urethane.

α_1-Adrenoceptor assays

Crude membranes from right ventricles were prepared as previously described (Deng *et al.*, 1996). Briefly, the right ventricles were homogenized in 8 ml ice-cold buffer A (Tris HCl 25 mM, EDTA 1 mM, $MgCl_2$ 2 mM and KCl 100 mM, pH 7.4) by means of a Polytron (at speed 10, 3 × 10 s). The homogenates were filtered through 4 layers of cheese cloth and centrifuged twice at 50,000 × *g* for 30 min at 4°C. The resulting pellets were washed with the incubation buffer (Tris HCl

[1] Author for correspondence at: Department of Pharmacology & Therapeutics, McGill University, McIntyre Medical Bldg., 3655 Drummond Street, Montreal, Que., Canada H3G 1Y6.

50 mM, $MgCl_2$ 2 mM and EDTA 1 mM, pH 7.4) and recentrifuged. For receptor assays, five ventricular membrane preparations were pretreated with 10 μM CEC at 37°C for 20 min. Membranes from prostate and salivary gland were prepared in the same way as myocardial membranes except that potassium chloride was not included in the homogenization buffer. Partially purified liver membranes were prepared by use of a discontinuous sucrose gradient according to the procedure described in detail by Wolfe *et al.* (1976). The final pellets were suspended in ice-cold incubation buffer to yield 0.1–0.18 mg protein ml^{-1}. Aortae from 12–15 rats were removed for one experiment and partially purified membranes were prepared by discontinuous sucrose gradient as described by Jagadeesh & Deth (1987). Proteins were determined by the dye-binding method with bovine serum albumin as the standard.

Aliquots (160 μl) of membrane suspension (15 to 20 μg protein/well) were added to 96 wells of a MultiScreen Assay System (Millipore, Bedford, MA, U.S.A.); 600 pM [^{3}H]-prazosin in the absence and the presence of increasing concentrations of BMY 7378 or 5-MU were added to the final incubation volume of 200 μl and were incubated for 60 min at room temperature; the nonspecific binding was determined by the addition of 10 μM phentolamine. The reaction was terminated by rapid filtration by means of a vacuum manifold. The filters (45 μm pore size) were washed three times with 100 μl of ice-cold incubation buffer and completely dried by a hair drier. The filters were then punched into 7 ml minivials. The membrane-bound radioactivity retained on the filters was counted in an LKB 1219 Rackbeta counter (Wallac) with a counting efficiency of approximately 45%. The binding data were analysed by use of a ReceptorFit Competition program (Lundon, Chagrin Falls, Ohio, U.S.A.).

Inotropic responses

Positive inotropic effects of phenylephrine in the presence of 1 μM propranolol (30 min contact) were determined on right ventricular, left atrial and left ventricular papillary muscles as described previously (Deng *et al.*, 1996). Right ventricles were divided into three; two papillary muscles were excised from each left ventricle. Preparations were set up at 32°C in a tissue bath containing Krebs buffer of the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.18, KH_2PO_4 1.2, $NaHCO_3$ 25, dextrose 11 and EDTA 0.03. The buffer was bubbled with a mixture of 95% O_2 and 5% CO_2 and strips were stimulated at 1 Hz, 5 ms pulse duration and 1.5 times the threshold voltage (20–30 V). The tension was adjusted in each preparation to yield maximal basal isometric contractions, which were recorded by means of Grass force-displacement transducers (FT03C) on a Grass polygraph. Following equilibration for 45–60 min with buffer changes every 10–15 min, 1 μM propranolol was added to all preparations. In all studies, one of the preparations served as the control and to the other BMY 7378 was added; 30 min later concentration-response curves to phenylephrine were obtained.

Right ventricular inositol phosphates

Total inositol phosphates were measured as previously described (Deng *et al.*, 1996). Briefly, ventricular slices (0.5 mm thick) were prepared with a tissue chopper. The slices were first equilibrated at 37°C for 30 min in an atmosphere of 95% O_2 and 5% CO_2 and then incubated with myo[^{3}H]-inositol (10 $\mu Ci ml^{-1}$) for 2 h. Slices were then washed three times with fresh buffer and transferred to vials containing 0.26 ml fresh buffer and 10 mM LiCl. The incubation was continued for 30 min in the presence of 1 μM propranolol and 0, 0.1, 0.3 or 1 μM BMY 7378; following this 30 min period, 10 μM phenylephrine was added and 5 min later the reaction was stopped by the addition of 0.3 ml ice-cold 10% trichloroacetic acid (TCA). The mixture was left on ice for 20 min, homogenized and centrifuged; the aqueous phase containing inositol phosphates was washed 5 times with 10 ml diethyl ether to remove TCA and the pH was adjusted to 7.4. Samples were applied to columns containing 50% slurry of 100–200 mesh AG 1-X8 anion-exchange resin (Bio-Rad, Mississauga, Ontario). Inositol phosphates were eluted with 1M ammonium formate and the radioactivity counted with a high efficiency scintillation fluid (OptiPhase HiSafe, Fisher, Montreal, Quebec); the counting efficiency was 40%. The protein concentration in the homogenate pellet was determined.

Contractions of aortic strips, vas deferens, spleen and prostate

Four spirally-cut strips of thoracic aorta (approximately 2 × 15 mm), two strips of the longitudinally-cut spleen (30–35 mm long), both prostates (approximately 5 × 15 mm) and prostatic portions of both vas deferens (approximately 30 mm long) were set up for recording contractions as described above with the exception that preparations were not electrically stimulated, Ca^{2+} in the buffer was 2.5 mM and the temperature of the bath was 37°C. The resting tension on aortic strips and spleen was 2 g, on prostate 1 g and on vas deferens 0.5 g. One of the four aortic strips and one of the two preparations of the spleen, prostate and vas deferens were used to construct control concentration-response curves to phenylephrine, BMY 7378 (30 min contact) was added to the other preparations from the same animal. For purposes of comparison, antagonistic activities of 5-MU on aorta, vas deferens and prostate and of CEC (100 μM for 30 min followed by a wash) on spleen were also determined; in these studies also, concentration-response curves to phenylephrine in the absence and presence of antagonists were determined on separate preparations from the same animal. Two concentration-response curves were not constructed on the same tissue because even after prolonged periods (> 1 h) of wash the tension did not reach the control base line in preparations of the spleen and the prostate.

Effects of BMY 7378 on blood pressure

Male rats (350–400 g) were anaesthetized with intraperitoneal injection of 1 g kg^{-1} urethane. Left jugular vein was cannulated for injections and left common carotid artery for recording blood pressure by means of a Statham pressure transducer (P23XL) on a Grass polygraph. Approximately one hour after the cannulation, increasing doses of BMY 7378 were injected; the next higher dose was injected after the response to the preceding dose had reached a plateau. At the end of the last injection of BMY 7378, the effect of 28 μmol kg^{-1} hexamethonium was determined.

Antagonist potencies

The potency of the agonist was expressed as pD_2 the negative log of the molar concentration of the agonist producing 50% of the maximal effect. The antagonist potency was expressed as pA_2 (Arunlakshana & Schild, 1959) when more than one concentration of the antagonist was used on preparations from the same animal (right ventricles and aorta) and the slope was not significantly different from unity. When only one concentration of the antagonist was used (papillary muscles, left atria, vas deferens and spleen), its antagonistic potency was expressed as pK_B; pK_B is the negative log of the dissociation constant K_B, which equals the molar concentration of the antagonist divided by the dose-ratio minus one (Besse & Furchgott, 1976).

Chemicals

The following agents were purchased: 8-[2-[4-(2-methyoxyphenyl)-1-piperazinyl]-8-azaspiro[4.5]decane-7.9-dione dihydrochloride (BMY 7378), 5-methylurapidil (5-MU) and chloroethylclonidine (CEC) (Research Biochemicals, Natick, MA, U.S.A.); hexamethonium bromide, phenylephrine HCl, phentolamine hydrochloride, (±)L-propranolol hydrochloride

and urethane (Sigma Chemical Co., St. Louis, MO, U.S.A.); [^{3}H]-prazosin (75–80 Cimmol^{-1}) and myo[^{3}H]-inositol (20.5 Ci mmol^{-1}) (NEN, Mississauga, Ontario, Canada); all other high purity chemicals were from Fisher Scientific (Montreal, Quebec, Canada).

Statistics

Data were compared by Student's t test for paired or unpaired data. A probability of less than 0.05 was assumed to denote a significant difference. Data are presented as means ± s.e.mean.

Results

Inhibition of [^{3}H]-prazosin binding

BMY 7378 inhibited the binding of [^{3}H]-prazosin to right ventricular membranes (Figure 1a; $n=6$); the inhibition of binding was also observed in membranes treated with CEC (Table 1; $n=5$). Nonlinear regression analysis of the inhibition curves for BMY 7378 in both the CEC-untreated and CEC-treated membrane preparations best fitted a one-site model. In contrast, inhibition curves with 5-MU fitted a two-site model in the absence of CEC treatment (Figure 1a; $n=5$) and a one-site model after treatment with CEC (Table 1; $n=5$). CEC treatment slightly but significantly ($P<0.05$) reduced the inhibitory potency of BMY 7378 against [^{3}H]-prazosin binding (Table 1).

The displacement curves of [^{3}H]-prazosin binding by BMY 7378 on partially purified aortic membrane preparations ($n=3$) best fitted a two-site model (Figure 1b); the pK_i of BMY 7378 for high affinity binding was 9.8 ± 0.4 and approximately 100 fold higher than on ventricular membranes and 1000 fold higher than on prostate, salivary gland, vas deferens and liver (Table 1). Apparent B_{max} of α_{1D} high affinity binding sites in the aorta (based on K_d for prazosin: 0.55 ± 0.09 nM) was 452 ± 79 fmol mg^{-1} protein.

BMY 7378 was approximately 10 fold less potent in inhibiting the binding of [^{3}H]-prazosin to membrane preparations of prostates (Figure 2a; $n=5$), salivary glands (Figure 2b; $n=5$), vas deferens (Figure 2c; $n=5$) and liver (Figure 2d; $n=4$) than it was in right ventricular membrane preparations (Table 1). Unlike the ventricular membrane preparations, the displacement curves of [^{3}H]-prazosin binding by 5-MU on prostate (Figure 2a) and salivary glands (Figure 2b) fitted a single site model; the Hill slopes of the displacement curves on membrane preparations of the prostates and salivary glands were 0.69 ± 0.12 and 0.71 ± 0.14, respectively.

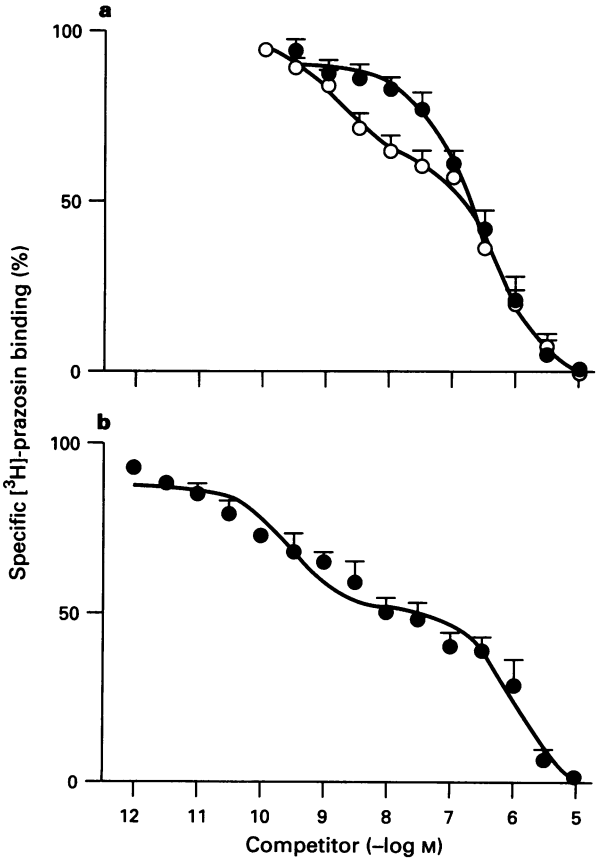

Figure 1 Inhibition of specific binding of [^{3}H]-prazosin to membrane preparations of right ventricles (a) and aorta (b) from rats by BMY 7378 (●) and 5-methylurapidil (○). Data are means ± s.e.mean (vertical lines) of 6 experiments with right ventricles and 3 experiments with aorta; aortae from 12 to 15 rats were used to prepare partially purified membranes for one experiment.

Antagonism against the inotropic effects of phenylephrine

BMY 7378 exerted no apparent effect on myocardial contractions up to a concentration of 1 μM; however, it inhibited the positive inotropic effects of phenylephrine on the right ventricular strips (Figure 3; $n=8$), papillary muscles (Table 2; $n=5$) and left atria (Table 2; $n=5$). The pK_B of BMY 7378 (300 nM, 30 min contact) was 6.87 ± 0.1 on the right ventricles, 6.89 ± 0.1 on papillary muscles and 7.02 ± 0.18 on left atria (Table 2). These pK_B values were almost identical with the pA_2 value of 7.0 ± 0.11, derived from the Schild plot (Figure 3b, slope 0.96 ± 0.15) on right ventricular strips. The pK_B of BMY 7378 on CEC (10 μM)-treated right ventricular strips was 6.55 ± 0.11 ($n=6$) and different ($P<0.05$) from the pA_2 (7.0 ± 0.11) on control strips.

Inhibition of phenylephrine effects on inositol phosphate production by right ventricles

BMY 7378 inhibited the phenylephrine-stimulated increases in inositol phosphates in a dose-dependent manner; the inhibition was approximately 50% at 100 nM and 95% at 1 μM BMY 7378 (Figure 4, $n=5$).

Table 1 Inhibitory potencies of BMY 7378 and 5-methylurapidil against [^{3}H]-prazosin binding to membrane preparations of different rat tissues

	BMY 7378		*5-Methylurapidil*	
Tissues	*pK_{iH}*	*pK_{iL}*	*pK_{iH}*	*pK_{iL}*
Right ventricle	–	–	9.22 ± 0.15	6.60 ± 0.15
Aorta	9.80 ± 0.40	6.23 ± 0.12	not done	
	pK_i		*pK_i*	
Right ventricle	7.47 ± 0.11		–	
Right ventricle (CEC)	6.95 ± 0.13[a]		8.60 ± 0.18[c]	
Prostate	6.62 ± 0.39[b]		8.41 ± 0.43[c]	
Van deferens	6.67 ± 0.15[b]		not done	
Salivary gland	6.46 ± 0.38[b]		8.31 ± 0.12[c]	
Liver	6.58 ± 0.06[b]		not done	

(CEC) indicates treatment of right ventricular membranes with 10 μM chloroethylclonidine for 20 min at 37°C; CEC was washed out before the binding studies were performed. pK_i is the negative log of K_i: the high affinity binding is expressed as pK_{iH}, low affinity as pK_{iL} and single site binding as pK_i. Values are mean ± s.e.mean of five to seven separate experiments. [a]Different ($P<0.05$) from the top values; [b]different ($P<0.01$) from values for right ventricles [c]different ($P<0.01$) from the value on the left in the same row and from the topmost value in the same column.

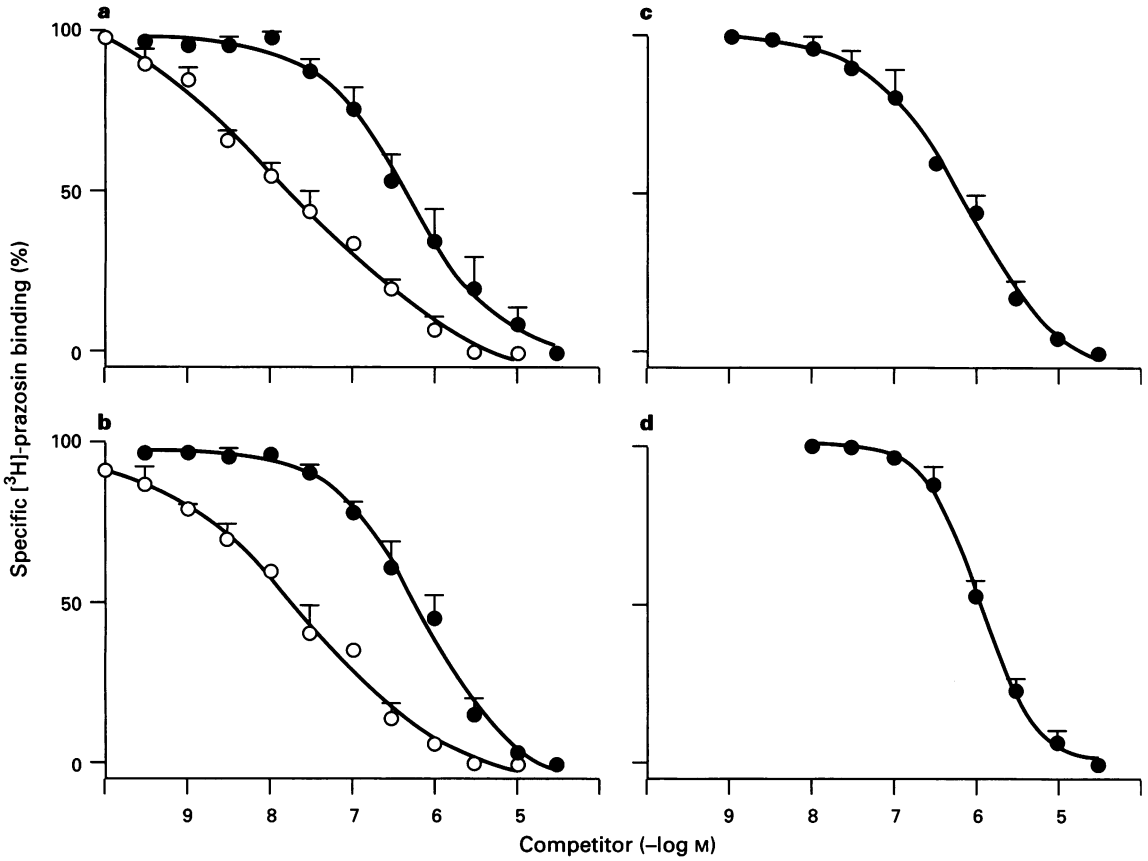

Figure 2 Inhibition of specific binding of [^{3}H]-prazosin by BMY 7378 (●) and 5-methylurapidil (○) in membrane preparations of rat prostate (a), salivary glands (b), vas deferens (c) and liver (d). Data are means ± s.e.mean (vertical lines) of 4–5 separate experiments.

Antagonism against phenylephrine on aortic strip

BMY 7378 caused a concentration-dependent inhibition of the phenylephrine-induced contractions of aortic strips (Figure 5a; $n=6$) yielding a pA_2 of 9.0 ± 0.14; the slope of the Schild plot was 1.05 ± 0.15 (Figure 5c). BMY 7378 was 100 fold more potent in antagonizing phenylephrine on the aorta than on right ventricular strips (Table 2). The α_{1A}-adrenoceptor selective antagonist 5-MU also caused a concentration-dependent antagonism against the effects of phenylephrine on aortic strips (Figure 5b; $n=5$) yielding a pA_2 of 7.98 ± 0.08 (Figure 5c); 5-MU was approximately 10 fold less potent than BMY 7378 as an antagonist against phenylephrine on the aortic strip.

Effects of BMY 7378 on responses of the spleen, prostate and vas deferens to phenylephrine

BMY 7378 antagonized the effects of phenylephrine on the spleen (Figure 6a; $n=6$) and vas deferens (Figure 6e; $n=5$) but not on the prostate (Figure 6c; $n=5$) The pK_B values of BMY 7378 on the spleen and vas deferens were 7.16 ± 0.21 and 7.17 ± 0.08, respectively, and very similar to that on myocardial preparations (Table 2). CEC (100 μM, 30 min contact followed by 30 min wash) shifted the phenylephrine concentration-response curve on the spleen to the right and significantly ($P<0.001$) suppressed the maximal effect from a control value of 390 ± 20 mg to 135 ± 10 mg ($n=5$). The pK_B of BMY 7378 against phenylephrine on splenic strips treated with 100 μM CEC was 7.03 ± 0.17 ($n=5$) and not different from its pK_B (7.16 ± 0.21) in the absence of CEC treatment. In contrast to BMY 7378, 5-MU potently inhibited the effects of phenylephrine on the prostate (Figure 6d; pK_B 8.85 ± 0.21; $n=5$) and vas deferens (Figure 6f; pK_B 8.89 ± 0.07; $n=5$); 5-MU did not antagonize the effects of phenylephrine on the spleen (Figure 6b). Also, 5-MU was an approximately 10 fold more potent antagonist of phenylephrine on the vas deferens (pK_B 8.89 ± 0.07) and prostate (pK_B 8.85 ± 0.21) than on aortic strips (pK_B 7.98 ± 0.08).

Hypotensive activity of BMY 7378

In urethane-anaesthetized normotensive rats ($n=5$), BMY 7378 produced a dose-dependent decrease in mean blood pressure (Figure 7). Hexamethonium (28 μmol kg^{-1}) did not produce a further decrease in blood pressure following the maximally effective hypotensive dose (100 nmol kg^{-1}) of BMY 7378 (Figure 7). At lower doses of BMY 7378 (0.01 and 0.1 nmol kg^{-1}), the hypotensive effect was preceded by an hypertensive (10–15 mmHg) effect.

Discussion

The primary objective of this study was to characterize the functional role of α_{1D}-adrenoceptors in rat tissues, which respond to α_1-adrenoceptor agonists or express different α_1 subtypes. Since we initiated these studies an important role of the α_{1D}-adrenoceptor in rat aorta, as found in the present study, has been documented by other investigators (Kenny *et al.*, 1995; Piascik *et al.*, 1995). To our knowledge, however, the present study in which BMY 7378 was used as an antagonist provides for the first time information on the functions of α_{1D}-adrenoceptors in the myocardium, spleen, vas deferens and prostate; the study also compliments the functional studies on aorta with binding data and demonstrates the hypotensive activity of BMY 7378.

BMY 7378 has been shown to be a selective α_{1D}-adrenoceptor antagonist (Goetz *et al.*, 1995; Kenny *et al.*,

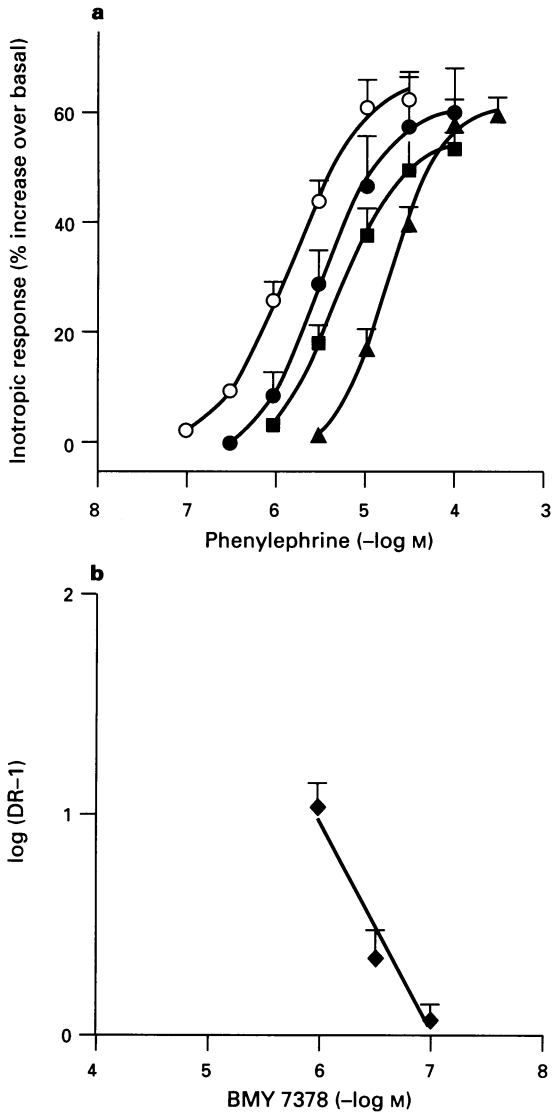

Figure 3 Concentration-inotropic response curves to phenylephrine on electrically stimulated (1 Hz) right ventricular strips of rats in the presence of different concentrations of BMY 7378 (a) and the Schild plot of the data (b). Thirty min before the construction of dose-response curves to phenylephrine, 1 μM propranolol and the following concentrations of BMY 7378 were added into the bath: 0 μM (○); 0.1 μM (●), 0.3 μM (■) and 1.0 μM (▲). Data are means ± s.e.mean (vertical lines) of 8 experiments. The slope of the Schild plot was not different from unity. Before the addition of phenylephrine, the contractile force was as follows (mg): control 552 ± 72; 0.1 μM BMY 7378, 450 ± 90; 0.3 μM BMY 7378, 575 ± 77; 1 μM BMY 7378, 512 ± 88.

1995). For example, in fibroblasts overexpressing cloned non-human α_1-adrenoceptor subtypes, BMY 7378 possesses 100 to 125 fold higher affinity for α_{1D}-adrenoceptors than for α_{1B}- and α_{1A}-adrenoceptors (Goetz *et al.*, 1995). Likewise in cells expressing cloned human α_1-adrenoceptors, the affinity of BMY 7378 for the α_{1D} subtype is 32 to 160 fold higher than that for the α_{1B} subtype and 100 to 630 fold greater than that for the α_{1A} subtype (Goetz *et al.*, 1995; Kenny *et al.*, 1995). Although the affinities of BMY 7378 for different native α_1 subtypes might not be as different as for overexpressed cloned receptors, as suggested by the binding data of this study (Table 1), it is still reasonable to assume that the main effects of BMY 7378 are produced by blockade of α_{1D}-adrenoceptors. At the same time a markedly lower antagonistic potency of BMY 7378 on heart, vas deferens and spleen than on aorta does not lead to unequivocal inference on the role of α_{1D}-adrenoceptors on these tissues. Furthermore, the present studies do not rule out possible antagonistic activities of BMY 7378 on α_{1B}-adrenoceptors, which constitute nearly 80% of the total α_1-adrenoceptors in the myocardium (Michel *et al.*, 1994a; Deng *et al.*, 1996) and a significant proportion in the spleen (Aboud *et al.*, 1993; Burt *et al.*, 1995; Scofield *et al.*, 1995). Since CEC can inactivate over 50% of the α_{1D}-adrenoceptors (Perez *et al.*, 1991; Laz *et al.*, 1994), it is difficult to study the functions of α_{1D}-adrenoceptors after selectively inactivating α_{1B}-adrenoceptors. The data of this study are discussed within these limitations.

BMY 7378 inhibited [^{3}H]-prazosin binding to right ventricular membranes (Table 1), antagonized the inotropic effects of phenylephrine (Table 2) and inhibited the phenylephrine-induced increase in PI turnover (Figure 4). Almost identical antagonist potencies of BMY 7378 against the inotropic effects of phenylephrine on the right ventricular strips, papillary muscles and left atria (Table 2) suggest interactions with same α_1 subtype in different regions of the myocardium. In addition, the inhibitory potency of BMY 7378 against [^{3}H]-prazosin binding to right ventricular membrane preparations (pK_i 7.47 ± 0.1) was very close to its pA_2 value (7.0 ± 0.11) against the inotropic effects of phenylephrine on this tissue; this would also suggest that both these effects of BMY 7378 were exerted via interactions with the same $\alpha_1$subtype, possibly α_{1D}-adrenoceptors. Furthermore, BMY 7378 could inhibit the binding of [^{3}H]-prazosin even after treatment of tissues with CEC. Also, BMY 7378 was approximately 10 fold more potent in inhibiting the [^{3}H]-prazosin binding to right ventricular membrane preparations than to membrane preparations from prostate and salivary gland, which primarily contain α_{1A}-adrenoceptors (Michel *et al.*, 1989; Yazawa & Honda, 1993) as well as to liver membranes, which contain α_{1B}-adrenoceptors (Han *et al.*, 1987; Faure *et al.*, 1994). It should nevertheless be emphasized that although α_{1D}-adrenoceptor mRNA is expressed in rat myocardium (Perez *et al.*, 1994; Price *et al.*, 1994; Rokosh *et al.*, 1994; Stewart *et al.*, 1994), it represents less than 5% of the total α_1-adrenoceptor mRNA (Scofield *et al.*, 1995) and the inotropic effects of the α_1 agonists are mainly produced via α_{1A}- and α_{1B}-adrenoceptors (Michel *et al.*, 1994b; Deng *et al.*, 1996). Taking into account these data, findings of the present study suggest a minor role for myocardial α_{1D}-adrenoceptors in the inotropic responses to α_1 agonists. This inference is consistent with a lower inhibitory potency of BMY 7378 against [^{3}H]-prazosin binding to the right ventricular membranes than to aortic membrane preparations (Table 1) and to overexpressed cloned α_{1D}-adrenoceptors in fibroblasts (Goetz *et al.*, 1995; Kenny *et al.*, 1995).

An important functional role for α_{1D}-adrenoceptors in rat aorta has recently been suggested by several investigators (Goetz *et al.*, 1995; Kenny *et al.*, 1995; Piascik *et al.*, 1995; Testa *et al.*, 1995). α_{1D}-adrenoceptor mRNA comprises over 85% of the total α_1 mRNA in rat aorta (Scofield *et al.*, 1995). Data of the present study are in accordance with these results. The pA_2 value of BMY 7378 against phenylephrine on aortic strips was 9.0 ± 0.14 and very close to its high affinity pK_i 9.8 ± 0.4. Moreover the antagonistic potency of BMY 7378 against phenylephrine on aortic strips was approximately 10 fold greater than that of 5-MU and 100 fold greater than its own antagonistic activity on heart, spleen and vas deferens.

Different blood vessels of the rat express α_{1D}-adrenoceptor mRNA in varying proportions and antagonistic activities of BMY 7378 on aorta and iliac arteries are higher than caudal, renal and mesenteric arteries (Piascik *et al.*, 1995); regardless of this variability , BMY 7378 would be expected to lower blood pressure. Although our studies do not identify the mechanism, the potent hypotensive action of BMY 7378 (Figure 7) is consistent with its ability to block α_{1D}-adrenoceptors. Our data do suggest that a selective α_{1D} antagonist devoid of interactions at 5-HT or other classes of receptors could prove to be a clinically useful antihypertensive agent.

The effects of phenylephrine on the prostate were antagonized by 5-MU but not by BMY 7378. This is consistent with

Table 2 Antagonist potencies of BMY 7378 against phenylephrine on different rat tissues

Tissues	*Control* pD_2	*BMY 7378* pD_2	*Dose-ratio*	*BMY 7378* pK_B
Right ventricle	5.80±0.07	5.32±0.06[a]	3.64±0.06	6.87±0.10
Papillary muscle	5.85±0.13	5.26±0.15[a]	3.64±0.70	6.89±0.10
Left atrium	5.55±0.07	4.93±0.15[a]	4.17±1.00	7.04±0.18
Aorta	8.47±0.13	6.96±0.05[a]	27.5±5.10[b]	9.00±0.13[b]
Vas deferens	5.60±0.19	4.73±0.22[a]	8.16±1.75	7.17±0.08
Spleen	5.26±0.16	4.61±0.05[a]	5.65±1.87	7.16±0.21
Spleen+CEC	4.33±0.11[c]	3.67±0.15[a,c]	5.57±1.45	7.03±0.17
Prostate	6.10±0.25	5.98±0.15	1.80±0.51[b]	Not determined

The concentration of BMY 7378 was 30 nM in aortic strips and 300 nM in all other tissues (30 min contract); myocardial preparations were stimulated at 1 Hz and 1 μM propranolol was added 30 min before the inotropic effects of phenyleprine were studied. pD_2 is the negative log of the molar concentration of phenyleprine producing 50% of the maximal effect. pK_B is the negative log of K_B (concentration of the antagonist divided by the dose-ratio minus 1). Values are mean±s.e.mean of five to eight separate experiments. [a]Different ($P<0.05$) from the control pD_2; [b]different ($P<0.01$) from all other values in the column; [c]different ($P<0.01$) from the immediate top value in the absence of CEC (chloroethylclonidine).

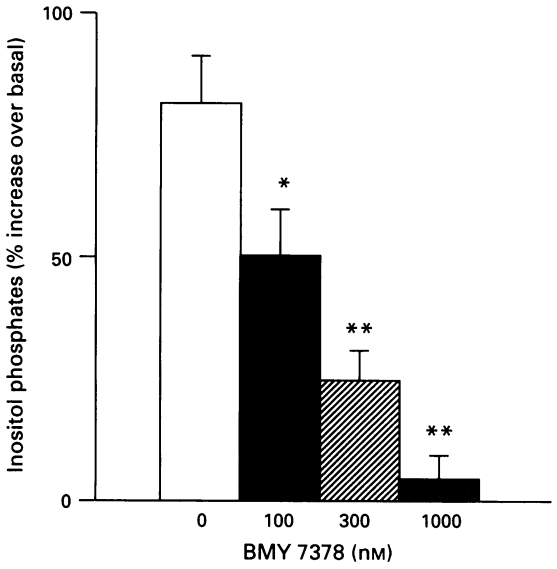

Figure 4 Inhibition by BMY 7378 of phenylephrine-induced increase in total inositol phosphates in right ventricular slices of rats. Propranolol (1 μM) and indicated concentrations of BMY 7378 were added 30 min before the addition of 10 μM phenylephrine. The basal level inositol phosphates was 470±17 d.p.m. mg^{-1} protein. Data are means±s.e.mean (vertical lines) of 5 separate experiments; Difference from the value in the absence of BMY 7378: * $P<0.05$; ** $P<0.01$.

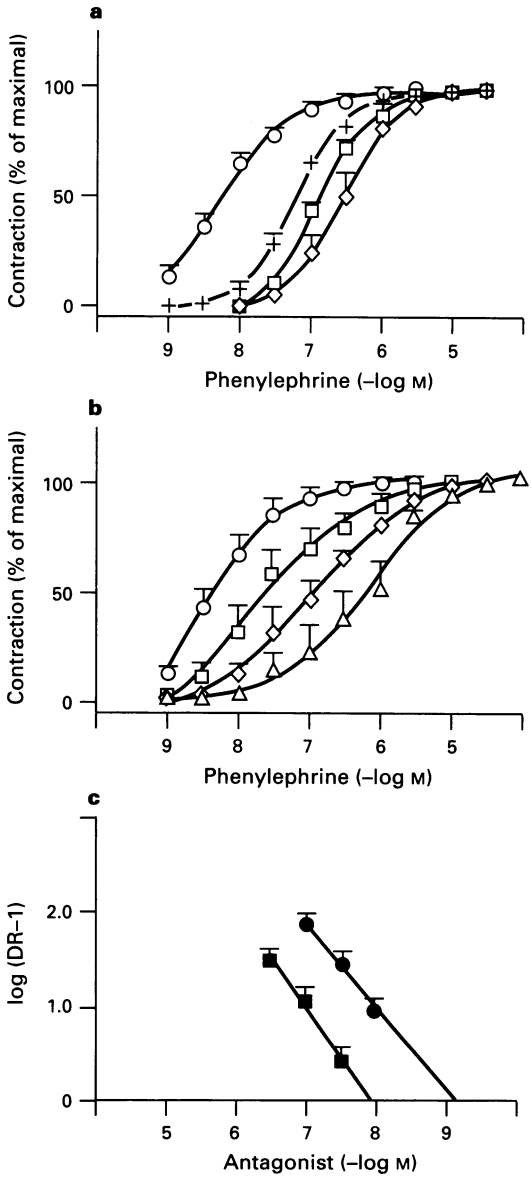

Figure 5 Inhibition of the phenylephrine-mediated contractions of rat aortic strips by BMY 7378 (a) and 5-methylurapidil (b) and the Schild plots of antagonistic activities (c). Thirty minutes before the concentration-response curves were constructed to phenylephrine, BMY 7378 or 5-methylurapidil was added into the bath at the following concentrations (nM): 0 (○); 10 (+); 30 (□); 100 (◇); 300 (△). The Schild plots of data (c): 5-methylurapidil (■); BMY 7378 (●). Data are means±s.e.mean (vertical lines) of 5–6 experiments.

studies showing that the rat prostate mainly expresses α_{1A}-adrenoceptor mRNA (Price *et al.*, 1994; Rokosh *et al.*, 1994) and virtually no α_{1D}-adrenoceptor mRNA (Scofield *et al.*, 1995); these findings also suggest that BMY 7378 exerts minimal effects on α_{1A}-adrenoceptors. On the other hand, the effects of phenylephrine on splenic strips were antagonized by BMY 7378 and not by 5-MU. These data are compatible with the finding that spleen expresses mRNAs for both α_{1D}- and α_{1B}- but not for α_{1A}-adrenoceptors (Faure *et al.*, 1994; Laz *et al.*, 1994; Perez *et al.*, 1994; Price *et al.*, 1994; Rokosh *et al.*, 1994). On the other hand, Scofield *et al.* (1995) detected mRNA for all the three α_1 subtypes in the spleen with α_{1D}-adrenoceptor mRNA being the most abundant; despite this relative distribution of α_1-adrenoceptor mRNAs, we found BMY 7378 to be a weak antagonist of phenylephrine on the spleen whereas CEC was quite effective. The almost identical antagonistic potencies of BMY 7378 against phenylephrine on the heart, vas deferens and spleen, and the ability of BMY 7378 to antagonize the effects of phenylephrine on spleen treated with 100 μM CEC would suggest that α_{1D}-adrenoceptors may play some role in the spleen.

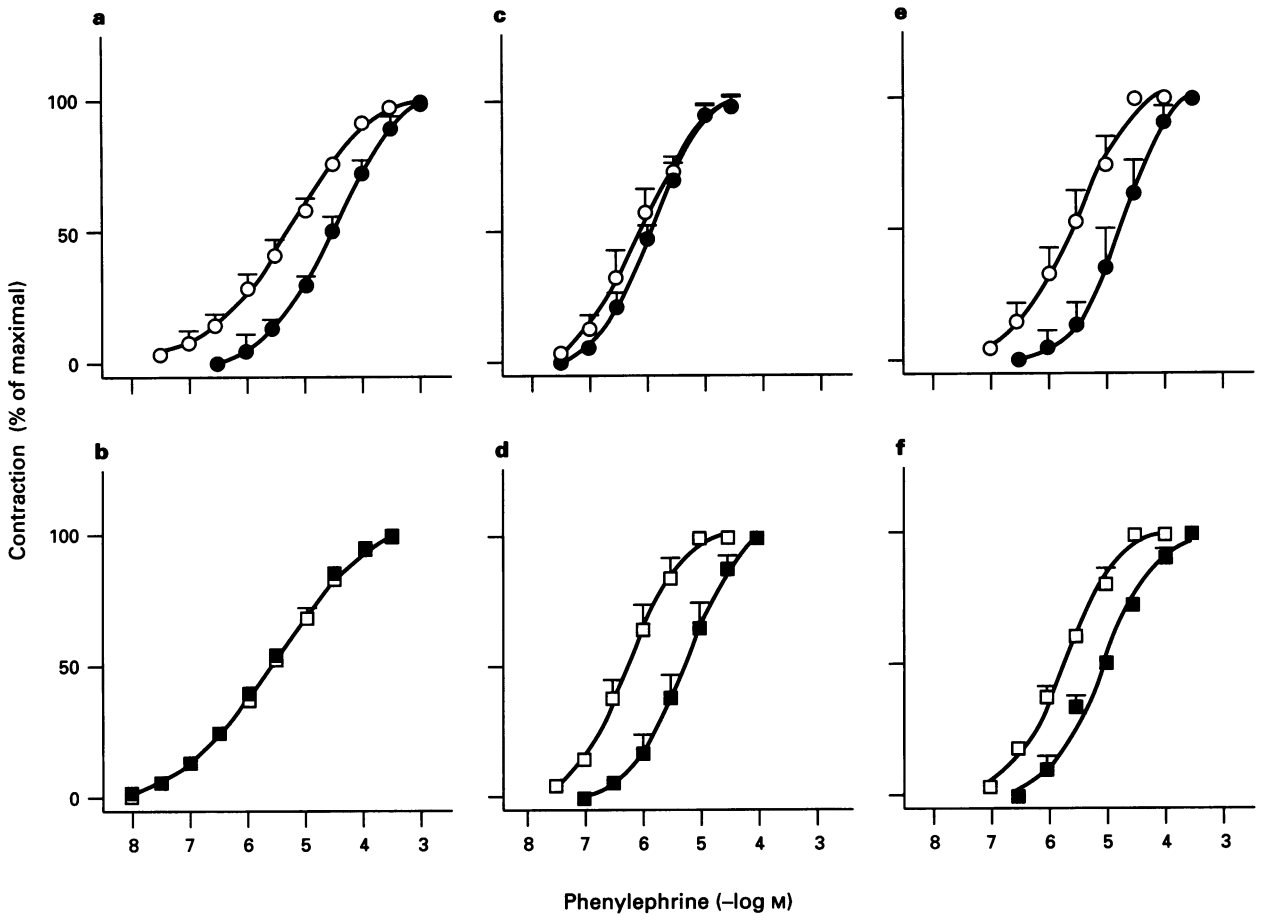

Figure 6 Effects of BMY 7378 (a, c, e) and 5-methylurapidil (5-MU; b, d, f) on the phenylephrine-induced contractions of rat spleen (a and b), prostate (c and d) and vas deferens (e and f); controls for BMY 7378 (○); controls for 5-MU (□); 300 nM BMY 7378 (●); 10 nM 5-methylurapidil (■). Antagonists were added 30 min before the construction of concentration-response curves to phenylephrine. Data are means ± s.e.mean (vertical lines) of 5–6 experiments.

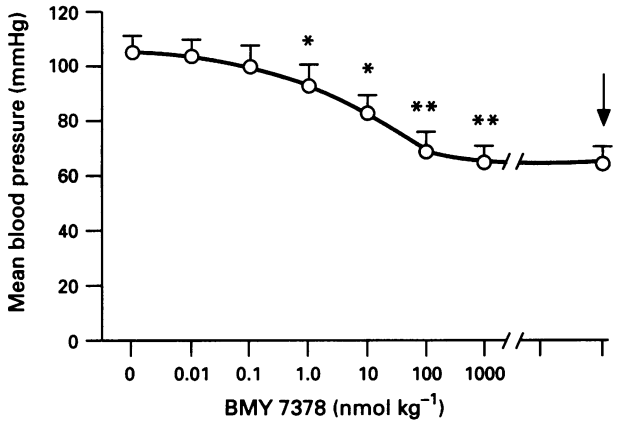

Figure 7 Effect of BMY 7378 on the mean blood pressure of urethane-anaesthetized rats. The arrow indicates the effect of 28 μmol kg^{-1} hexamethonium which was injected after the last dose of BMY 7378. Data are means ± s.e.mean (vertical lines) from 5 experiments; $^*P<0.05$, $^{**}P<0.01$ compared with the control mean blood pressure.

BMY 7378 was approximately 1,000 fold less effective in displacing [^{3}H]-prazosin bound to plasma membranes of vas deferens and prostate than of aorta. Moreover, BMY 7378 was a weak antagonist of phenylephrine on vas deferens and exerted no antagonist effect on prostate up to a concentration of 300 nM. These results would suggest that α_{1D}-adrenoceptors may have no role in the prostate and a minor role, if any, in vas deferens. It is nonetheless surprising that α_{1D}-adrenoceptor mRNA is slightly more abundant than α_{1A}-adrenoceptor mRNA in vas deferens (Scofield *et al.*, 1995) and yet the α_{1A} antagonist 5-MU is an extremely potent antagonist of phenylephrine on vas deferens (pK_B 8.89 ± 0.07) whereas BMY 7378 is a weak antagonist (pK_B 7.17 ± 0.08).

The present study was not designed to relate binding data and functional studies with α_{1D}-adrenoceptor mRNA expression. Nevertheless, our data suggest that a functional role of α_{1D}-adrenoceptors cannot be accurately predicted from the abundance of its mRNA in different tissues. For example, α_{1D}- and α_{1A}-adrenoceptor mRNAs in vas deferens are equally abundant (Scofield *et al.*, 1995) but 5-MU was 100 fold more potent than BMY 7378 in antagonizing the effects of phenylephrine. Likewise, α_{1D} mRNA is more abundant than α_{1A} mRNA and α_{1B} mRNA and the latter two are expressed in almost equal amounts in the spleen (Scofield *et al.*, 1995); however, CEC inhibited the maximal effects of phenylephrine on the spleen whereas BMY 7378 exerted little antagonism and 5-MU was ineffective.

In summary, the data of this study suggest an important role for α_{1D}-adrenoceptors in the rat aorta, a minor role in myocardium, spleen and vas deferens and virtually no role in the prostate. The results also point to an antihypertensive therapeutic potential of a selective α_{1D}-antagonist, which might less adversely affect male sexual functions than the currently used adrenoceptor blockers which are not selective for α_1-adrenoceptor subtypes.

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