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Buspirone functionally discriminates tissues endowed with α_1-adrenoceptor subtypes A, B, D and L

Manfrid Eltze *, Helga König, Brigitte Ullrich, Thomas Grebe

Department of Pharmacology, Byk Gulden, D-78467 Konstanz, Germany

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Abstract

The affinity for functional α_1-adrenoceptor subtypes of buspirone in comparison with its close structural analogs and selective α_{1D}-adrenoceptor antagonists, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione) and MDL 73005EF (8-[2-(1,4-benzodioxan-2-ylmethylamino)ethyl]-8-azaspiro[4.5]decane-7,9-dione), was determined, namely at subtype A in rat vas deferens and perfused kidney, at subtype B in guinea-pig and mouse spleen, at subtype L in rabbit spleen, and at subtype D in rat aorta and pulmonary artery against noradrenaline-evoked contractions. BMY 7378 and MDL 73005EF were confirmed as 30- and 20-fold selective antagonists, respectively, for α_{1D}- over both α_{1A}- and α_{1B}-adrenoceptors. Buspirone was a weak antagonist without intrinsic activity at α_{1A}-adrenoceptors in rat vas deferens ($pA_2 = 6.12$), at α_{1B}-adrenoceptors in guinea-pig and mouse spleen ($pA_2 = 5.54$ and 5.59) and at α_{1L}-adrenoceptors in rabbit spleen ($pA_2 = 4.99$), but caused partial vasoconstriction in rat kidney that was attenuable by the subtype D-selective adrenoceptor antagonist BMY 7378, but hardly by the subtype A-selective adrenoceptor antagonist B8805-033 ((±)-1,3,5-trimethyl-6-[[3-[4-((2,3-dihydro-2-hydroxymethyl)-1,4-benzodioxin-5-yl)-1-piperazinyl]propyl]amino]-2,4(1*H*,3*H*)-pyrimidinedione), confirming the additional presence of α_{1D}-adrenoceptors mediating rat renal vasoconstriction. Buspirone behaved as a partial agonist at α_{1D}-adrenoceptors in rat aorta ($pD_2 = 6.77$, intrinsic activity (i.a.) = 0.40) and pulmonary artery ($pD_2 = 7.16$, i.a. = 0.59). With buspirone as agonist in these tissues, the pA_2 values of subtype-discriminating antagonists were consistent with their α_{1D}-adrenoceptor affinity determined in rat aorta against noradrenaline and with published binding data on cloned α_{1d}-adrenoceptors. The results provide pharmacological evidence that (1) in functional preparations for the A subtype, like rat vas deferens and perfused kidney, for the B subtype, like guinea-pig and mouse spleen, and for the L subtype, like rabbit spleen, buspirone is a weak antagonist without intrinsic activity, but (2) behaves as a partial agonist in rat aorta and pulmonary artery as models for the D subtype and (3) detects an additional vasoconstrictor α_{1D}-adrenoceptor in rat kidney. Buspirone, like its close analogs BMY 7378 and MDL 73005EF, thus might also be a useful tool for functionally discriminating α_{1D}- from α_{1A}-, α_{1B}- and α_{1L}-adrenoceptors in various tissues.

Keywords: α_1-Adrenoceptor, subtypes A, B, D and L; Buspirone; Aorta; Pulmonary artery; Kidney; (Rat)

1. Introduction

α_1-Adrenoceptors comprise a heterogeneous family (Minneman and Esbenshade, 1994). Three natively expressed subtypes (α_{1A}, α_{1B} and α_{1D}, with uppercase letters) can be distinguished pharmacologically and exhibit equivalency to cloned and expressed α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors (with lowercase letters) in various tissues (Hieble et al., 1995). The native and cloned subtypes exhibit different affinities for selective antagonists, e.g., tamsulosin and 5-methyl-urapidil ($\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$), spiperone ($\alpha_{1B} > \alpha_{1A} = \alpha_{1D}$), or BMY 7378 and MDL 73005EF ($\alpha_{1D} > \alpha_{1A} = \alpha_{1B}$) (Han et al., 1987; Gross et al., 1988; Michel et al., 1989; Forray et al., 1994; Goetz et al., 1995; Saussy et al., 1996). This subdivision has been supported by the different sensitivity of α_1-adrenoceptor-mediated responses to Ca^{2+} channel antagonists and to the alkylating agent, chloroethylclonidine, which preferentially

* Corresponding author. Tel.: +49-7531-842617; fax: +49-7531-8492617; E-mail: manfrid.eltze@byk.gulden.de

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attenuate α_{1A}- and α_{1B}-adrenoceptor-mediated effects, respectively (Han et al., 1987; Perez et al., 1994). Additionally, a putatively fourth α_1-adrenoceptor with low affinity for prazosin ($pA_2 < 9.0$) and HV 723 ($pA_2 < 8.0$; α-ethyl-3,4,5-trimethoxy-α-(3-([2-methoxyphenoxy) ethyl] amino)propyl)benzeneacetonitrile), designated α_{1L}, has been identified in functional studies in blood vessels (Muramatsu et al., 1990b, 1998) and in tissues of the lower urinary tract (Chalmers et al., 1997; Testa et al., 1997), and is also suggested to mediate contraction of rabbit spleen (Oriowo, 1998). Convincing evidence has accumulated that native α_{1A}-adrenoceptors mediate smooth muscle contraction of rat vas deferens (Han et al., 1987; Eltze and Boer, 1992; Kenny et al., 1994) and vasoconstriction in rat perfused kidney (Eltze et al., 1991; Blue et al., 1995), whereas α_{1B}-adrenoceptors mediate contraction in guinea-pig and mouse splenic strips (Eltze, 1994, 1996). After a long controversy it is now undisputed that contraction of the rat thoracic aorta is mediated by stimulation of the α_{1D}-adrenoceptor subtype (Saussy et al., 1994; Kenny et al., 1995; Testa et al., 1995). Vasoconstrictor α_{1D}-adrenoceptors have also been detected in rat pulmonary artery (Hussain and Marshall, 1997) and in rat renal artery (Villalobos-Molina et al., 1997).

BMY 7378 was the first selective antagonist at α_{1D}-adrenoceptors (Saussy et al., 1994; Goetz et al., 1995). Recently, another substituted 8-azaspiro[4,5]decane-7,9-dione, MDL 73005EF, was found to have significant selectivity for the α_{1D}-adrenoceptor (Saussy et al., 1996). Previous studies with the 5-HT_{1A} receptor agonist, buspirone, which differs only in its arylpiperazinyl moiety from its analog BMY 7378 (Fig. 1), have shown it to have both partial agonist ($pD_2 = 5.5–6.4$, i.a. = 0.3–0.5) and antagonist properties ($pA_2 = 5.6–5.8$) against phenylephrine-induced contractions at α_1-adrenoceptors of rabbit aorta (Rimele et al., 1987; Gürdal et al., 1992; Castillo et al., 1993). The subtype predominantly involved in contraction in this tissue appears as yet unresolved and contraction is probably mediated by more than one distinct subtype (Muramatsu et al., 1990a, 1998: α_{1B} and α_{1L}; Suzuki et al., 1990; Oriowo and Ruffolo, 1992; Castillo et al., 1993: α_{1A} and α_{1B}; Leonardi et al., 1997: α_{1D}; Testa et al., 1997: α_{1L}). The contractile effects of buspirone were also observed in rat thoracic aorta ($pD_2 = 6.7–7.2$, i.a. = 0.5; Rimele et al., 1987; Saussy et al., 1996) and in vivo by eliciting pressor responses after i.v. administration in rats and dogs (Hanson et al., 1986), by increasing blood pressure in pithed rats (Castillo et al., 1995), and by decreasing external carotid blood flow in anesthetized dogs (Terrón et al., 1996), an effect that is susceptible to blockade by prazosin. However, although some binding data for cloned α_1-adrenoceptor subtypes (pK_i at $\alpha_{1a} = 5.3–5.9$, $\alpha_{1b} = 5.1–5.3$, $\alpha_{1d} = 6.0–6.6$; Saussy et al., 1996) have been published, functional affinity data for buspirone derived from experiments with isolated tissues possessing distinct α_1-adrenoceptor subtypes (A, B, D and L) are missing.

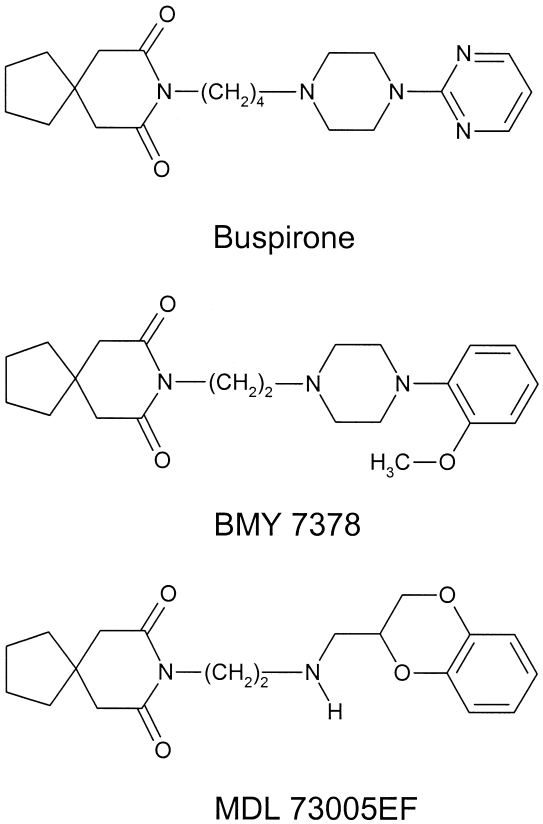

Fig. 1. The chemical structures of buspirone and its analogs investigated in this study.

The purpose of the present study was to evaluate the antagonist and possible agonist actions of buspirone in comparison to its close analogs, BMY 7378 and MDL 73005EF, on isolated tissue preparations known to be nearly exclusively supplied with α_1-adrenoceptors of the subtype A, namely rat vas deferens and perfused kidney, with the subtype B, namely guinea-pig and mouse splenic strips, with the subtype L recently suggested to mediate contraction of rabbit spleen (Oriowo, 1998), and with the subtype D, namely the rat thoracic aorta. Additionally, the rat pulmonary artery (Hussain and Marshall, 1997), a tissue with α_{1D}-adrenoceptors and therefore also suitable for investigating selective α_{1D}-adrenoceptor activation, was chosen to characterize the action of buspirone. The affinities of reference antagonists at α_1-adrenoceptor subtypes in some tissues have previously been published (Eltze and Boer, 1992; Eltze, 1994, 1996, 1997). The data obtained were compared to published binding affinities of the compounds at cloned α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors expressed in various tissues. The results presented here reveal that selective stimulation of α_{1D}-adrenoceptors by buspirone mediates partial vasoconstriction in rat aorta, pulmonary artery and perfused kidney, but not in tissues endowed with the A, B and L subtypes of α_1-adrenoceptors at which buspirone is a low-affinity antagonist.

2. Materials and methods

2.1. Rat vas deferens and perfused rat kidney: α_{1A}-adrenoceptors

Prostatic portions of vas deferens taken from Sprague–Dawley rats (180–250 g) were set up in 20 ml organ baths containing Tyrode solution plus 10^{-5} M cocaine, maintained at 37°C and gassed with a mixture of 95% O_2–5% CO_2. Cumulative concentration–response curves for isotonic contractions in response to cumulatively added noradrenaline (10^{-7}–10^{-4} M), whereby the rapidly developing phasic component of the contraction was measured, were generated in the absence or presence of different antagonist concentrations equilibrated with the tissue for 20 min (Eltze et al., 1991).

The potency of α_1-adrenoceptor antagonists to attenuate noradrenaline-evoked vasoconstriction was evaluated in isolated kidneys taken from male normotensive rats (Sprague–Dawley, 390–420 g) perfused via the abdominal aorta at a constant pressure of 100 cm H_2O with prewarmed Tyrode solution gassed with 95% O_2–5% CO_2. The prerenal perfusate flow was measured continuously using an electromagnetic flowmeter. After perfusion of the kidney without any drug for 30 min, during which vascular flow stabilized at 15.4 ± 1.2 ml/min (mean ± S.D., $n = 26$), the kidney was continuously perfused with 6×10^{-7} M noradrenaline, which reduced renal perfusion flow by about 70–80%. Once the vasoconstriction had stabilized, increasing doses of the test drugs (100 μl aqueous bolus) were injected within 2 s into the renal inflow tract and the resulting vasodilation was recorded. The decrease in renal flow obtained by perfusion of the kidney with noradrenaline in the absence of the test substance was taken as 100%, and the percent reversal of this effect following injection of increasing doses of the antagonist was calculated for determination of their half-maximal vasodilator effect ($-\log ED_{50}$; Eltze et al., 1991).

In a second series of experiments, the vasoconstrictor effect of injected buspirone was evaluated on the basal vascular tone of perfused kidneys and expressed as percent vasoconstriction. In experiments in which this effect was antagonized, a maximally effective dose of buspirone (10^{-6} mol) was injected together with appropriate doses of BMY 7378 (3×10^{-9}, 10^{-8} and 10^{-7} mol) or B8805-033 (10^{-7}, 3×10^{-7} and 10^{-6} mol) and the change in perfusion flow (ml/min) was calculated. Statistically significant differences between vasoconstriction elicited by buspirone alone and that in the presence of antagonist were evaluated by Student's *t*-test.

2.2. Guinea-pig and mouse spleen: α_{1B}-adrenoceptors

Spleens were obtained from male guinea-pigs (350–400 g, killed by a blow on the head and exsanguination) or male mice (25–30 g, previously anesthetized by a short exposure to isoflurane, Forene®, Abbott). The spleens were longitudinally cut into six and two strips, respectively, and were set up in 10 ml organ baths under a resting tension of 1.0 and 0.8 g, respectively, for recording isometric contractile responses in Krebs–Ringer bicarbonate buffer maintained at 37°C and gassed with 95% O_2–5% CO_2. The buffer additionally contained 3×10^{-7} M desipramine, 3×10^{-5} M corticosterone and 10^{-6} M propranolol. Isometric contractions in response to cumulative administration of noradrenaline (half-log unit steps from 10^{-7}–10^{-4} M in guinea-pig spleen; one-log unit steps from 10^{-8}–10^{-4} M in mouse spleen) were generated in the absence or presence of antagonists equilibrated with the splenic strips for 30 min (Eltze, 1994, 1996).

2.3. Rabbit spleen: putative α_{1L}-adrenoceptors

Similarly, longitudinal splenic strips (3×15 mm) taken from male New Zealand White rabbits (2.5–3.0 kg, previously anesthetized with pentobarbital sodium, 60 mg/kg i.v.) were set in organ baths containing the above-mentioned and modified Krebs–Ringer bicarbonate buffer used for guinea-pig and mouse spleen. Isometric contractions under a resting tension of 1 g were recorded for a number of agonists and were related to the maximal effect elicited by 3×10^{-5} M noradrenaline (100%). Since the α_2-adrenoceptor agonists, UK 14.304 (10^{-7}–10^{-5} M) and clonidine (10^{-6}–10^{-4} M), evoked splenic contractions of up to 33 and 27% of maximum, respectively, yohimbine (10^{-7} M) was additionally included in the nutrient solution to block α_2-adrenoceptors in antagonist experiments in which the agonist noradrenaline was used. Antagonist affinities were determined from cumulative concentration–response curves for noradrenaline (10^{-8}–3×10^{-5} M) in the absence and presence of antagonists equilibrated with the tissue for 30 min (Oriowo, 1998).

2.4. Rat thoracic aorta and pulmonary artery: α_{1D}-adrenoceptors

Ring preparations from the thoracic aorta and pulmonary artery of male rats (Sprague–Dawley, 350–400 g) were mounted in 10 ml organ baths under a resting tension of 1 g in Krebs–Ringer bicarbonate buffer maintained at 37°C and gassed with 95% O_2–5% CO_2. The buffer additionally contained 10^{-7} M desipramine, 3×10^{-5} M corticosterone, 10^{-6} M propranolol and 10^{-7} M yohimbine. Isometric contractions in response to cumulatively added noradrenaline or buspirone (10^{-8}–3×10^{-6} M) were generated in the absence or presence of antagonists equilibrated with the tissue for 30 min (Eltze and Boer, 1992). Some potency values for reference agonists (pD_2)

Table 1
Affinities (pA_2) and potencies ($-\log ED_{50}$) of α_1-adrenoceptor subtype-selective antagonists in comparison with buspirone at subtype A in rat vas deferens (RVD) and rat kidney (RK), at subtype B in guinea-pig spleen (GPS) and mouse spleen (MS), and at subtype D in rat aorta (RA). The pA_2 values (with slopes β of regression lines in parentheses) were calculated from constrained Schild plots ($\beta = 1$) for competitive antagonism at α_1-adrenoceptor subtypes in the different tissues. The results are presented as means ± S.E.M. of $n = 6–7$ for rat kidney and $n = 12–16$ for pA_2 or pD_2 determinations for each drug in the different tissues. Most data for the reference antagonists in rat vas deferens, kidney and aorta, guinea-pig and mouse spleen were taken from Eltze and Boer (1992) and Eltze (1994, 1996, 1997)

	Subtype A		Subtype B		Subtype D	
Tissue	RVD	RK	GPS	MS	RA	RA
Agonist	NA (pA_2)	NA ($-\log$ mol)	NA (pA_2)	NA (pA_2)	NA (pA_2)	Buspirone (pA_2)
Buspirone	6.12 ± 0.09 (0.94)	7.71 ± 0.25	5.54 ± 0.08 (0.77)[a]	5.59 ± 0.12 (1.03)	pD_2 = 6.77 ± 0.08 (i.a. = 0.40 ± 0.07)	
BMY 7378	6.67 ± 0.15 (0.93)	8.76 ± 0.19	6.55 ± 0.18 (1.02)	6.76 ± 0.07 (0.93)	8.15 ± 0.16 (1.00)	8.22 ± 0.09 (1.04)
MDL 73005EF	5.84 ± 0.08 (0.93)	8.08 ± 0.22	5.88 ± 0.24 (0.73)[a]	6.30 ± 0.09 (0.83)	7.23 ± 0.14 (1.01)	7.37 ± 0.05 (0.90)
5-Methyl-urapidil	9.10 ± 0.09 (1.06)	10.78 ± 0.16	6.95 ± 0.17 (0.91)	7.03 ± 0.07 (0.93)	7.46 ± 0.05 (0.89)	7.45 ± 0.06 (1.04)
Tamsulosin	10.24 ± 0.05 (1.19)	10.92 ± 0.11	8.33 ± 0.08 (1.03)	8.62 ± 0.17 (1.07)	9.56 ± 0.07 (0.97)	9.82 ± 0.19 (1.05)
Spiperone	7.63 ± 0.03 (0.93)	9.54 ± 0.12	8.05 ± 0.16 (0.77)[a]	8.29 ± 0.19 (0.91)	7.82 ± 0.08 (0.75)[a]	8.09 ± 0.06 (0.97)
Flesinoxan	6.99 ± 0.03 (1.00)	8.84 ± 0.14	5.70 ± 0.13 (0.77)[a]	5.54 ± 0.08 (0.97)	5.48 ± 0.06 (0.93)	6.10 ± 0.05 (0.98)
B8805-033	8.40 ± 0.11 (1.26)[a]	9.82 ± 0.13	5.21 ± 0.08 (1.05)	5.30 ± 0.08 (0.89)	5.24 ± 0.11 (0.85)	5.50 ± 0.11 (0.97)
Dapiprazole	7.93 ± 0.10 (1.02)	9.56 ± 0.17	7.13 ± 0.09 (0.97)	7.21 ± 0.16 (1.00)	8.26 ± 0.05 (1.07)	8.06 ± 0.19 (0.88)

[a] Slope significantly different from unity ($P < 0.05$).

in rat aorta were taken from the article by Eltze and Boer (1992).

2.5. Antagonist affinities, agonist potencies and linear regressions

In antagonist experiments, Schild plots were constructed to estimate the pA_2 value and the slope β of the regression line for each experimental series, which generally comprised at least three different antagonist concentrations (Arunlakshana and Schild, 1959). The pA_2 values quoted in Tables 1 and 2 were calculated from Schild plots in which the slopes of the regression lines were constrained to 1.00. In those cases where the slope of the Schild plot differed significantly from unity ($P < 0.05$), pA_2 values determined from constrained regression lines ($\beta = 1.00$) should be regarded as approximations.

The slope β of the regression line of data comparing two sets of antagonist affinities was calculated by the least-squares method. Calculation of the correlation coefficient r and a t-test for significance of the difference of the slope from unity were performed to test for receptor identity or non-identity in rat aorta using noradrenaline and buspirone as agonists against a series of subtype-discriminating antagonists. Linear regression was also used to correlate pA_2 values obtained in functional studies with pK_i values of the antagonists obtained in binding experiments with cloned α_1-adrenoceptor subtypes a, b and d,

Table 2
Comparison of pA_2 values calculated from constrained Schild plots (with slopes β of regression lines in parentheses) for a number of α_1-adrenoceptor subtype-discriminating antagonists determined in rat pulmonary artery against buspirone-evoked contractions with their published pK_i values for cloned and expressed α_1-adrenoceptor subtypes a, b and d. Given are means ± S.E.M. of $n = 8–12$ for rat pulmonary artery or for the number of binding data (indicated in brackets) taken from the literature. Binding data were compiled from published studies using, rat, hamster, bovine and human cloned α_1-adrenoceptor subtypes expressed transiently in a variety of cell lines, as previously summarized by Eltze (1996). The data for buspirone and MDL 73005EF at rat and hamster cloned α_1-adrenoceptor subtypes a, b and d were taken from Saussy et al. (1996)

	Rat pulmonary artery (pA_2)	Cloned adrenoceptors (pK_i)		
		α_{1a}	α_{1b}	α_{1d}
Buspirone	pD_2 = 7.16 ± 0.05 (i.a. = 0.59 ± 0.02)	5.66 (1)	5.11 (1)	6.04 (1)
Tamsulosin	9.35 ± 0.07 (0.90)	10.25 ± 0.3 (5)	8.97 ± 0.2 (5)	9.69 ± 0.3 (5)
BMY 7378	8.00 ± 0.09 (1.10)	6.28 ± 0.1 (5)	6.51 ± 0.2 (5)	8.44 ± 0.2 (5)
Spiperone	8.17 ± 0.07 (0.95)	7.87 ± 0.1 (9)	8.52 ± 0.2 (8)	7.96 ± 0.1 (8)
5-Methyl-urapidil	7.48 ± 0.11 (0.93)	8.66 ± 0.1 (13)	6.87 ± 0.1 (13)	7.46 ± 0.2 (14)
MDL 73005EF	7.32 ± 0.08 (0.86)	5.75 (1)	6.19 (1)	7.31 (1)
B8805-033	5.48 ± 0.07 (1.12)	8.70 (1)[a]	5.60 (1)[a]	< 6.00[b]

[a] Binding affinity at native subtypes A and B in rat cortex and rat liver, respectively (Eltze et al., 1996).
[b] Binding affinity at human cloned α_{1d}-adrenoceptors (Dr. R. Testa, personal communication).

the latter being taken from the literature and listed in Table 2.

The EC_{50} values of α_1-adrenoceptor agonists to evoke half-maximal contractions of rat aorta, rat pulmonary artery and rabbit spleen ($pD_2 = -\log EC_{50}$) were obtained by non-linear regression analysis of each individual response related to the maximal effect of noradrenaline (i.a. = 1.00) determined in the same tissue.

2.6. Drugs

Buspirone HCl, spiperone HCl, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione diHCl), MDL 73005EF (8-[2-(1,4-benzodioxan-2-ylmethylamino)ethyl]-8-azaspiro[4.5]decane-7,9-dione HCl) (RBI, Cologne, Germany). Flesinoxan HCl (Duphar, Weesp, The Netherlands). Tamsulosin HCl (Yamanouchi, Japan). Dapiprazole HCl (Winzer, Germany). HV 723 (α-ethyl-3,4,5-trimethoxy-α-(3-([2-(methoxyphenoxy) ethyl]amino)propyl)benzeneacetonitrile fumarate) was kindly provided by Prof. I. Muramatsu (Matsuoka, Japan). 5-Methyl-urapidil, B8805-033 ((±)-1,3,5-trimethyl-6-[[3-[4-((2,3-dihydro-2-hydroxymethyl)-1,4-benzodioxin-5-yl)-1-piperazinyl]propyl]amino]-2,4(1*H*,3*H*)-pyrimidinedione)(Byk Gulden). Phenylephrine HCl (Serva, Heidelberg, Germany). Methoxamine HCl (Wellcome, London, UK). Indanidine HCl (Sgd 101/75) (Siegfried, Zofingen, Switzerland). Cirazoline HCl (Synthelabo, Bagneux, France). (*R*)-A-61603 (*N*-[5-(4,5-dihydro-1*H*-imi-dazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methansulfonamide HBr)(Abbott Laboratories, IL, USA). SDZ NVI 085 ((−)-(4*aR*,10*aR*)-3,4,4*a*,5,10,10*a*-hexahydro-6-methoxy-4-methyl-9-methylthio-2*H*-naphth[2,3-*b*]-1,4-oxazine hydrogen malonate) (Novartis, formerly Sandoz, Basel, Switzerland). All other drugs were purchased from Sigma (Munich, Germany).

3. Results

3.1. α_{1A}-adrenoceptors: rat vas deferens and rat perfused kidney

Buspirone (10^{-6}–10^{-5} M; Fig. 2, top), BMY 7378 (3×10^{-7}–10^{-5} M) and MDL 73005EF (10^{-6}–3×10^{-5} M), equilibrated with the rat vas deferens for 20 min, shifted the noradrenaline concentration–response curves to the right, indicating competitive antagonism at α_{1A}-adrenoceptors, without eliciting an observable contractile response of their own in this tissue. The resulting pA_2 values obtained for the drugs from constrained Schild plots were 6.12, 6.67 and 5.84, respectively (Fig. 2, bottom; Table 1). The affinities of the reference antagonists, 5-methyl-urapidil, tamsulosin, spiperone, flesinoxan, dapiprazole and B8805-033, previously determined in this tissue (Eltze, 1994, 1996, 1997), are also included in Table 1.

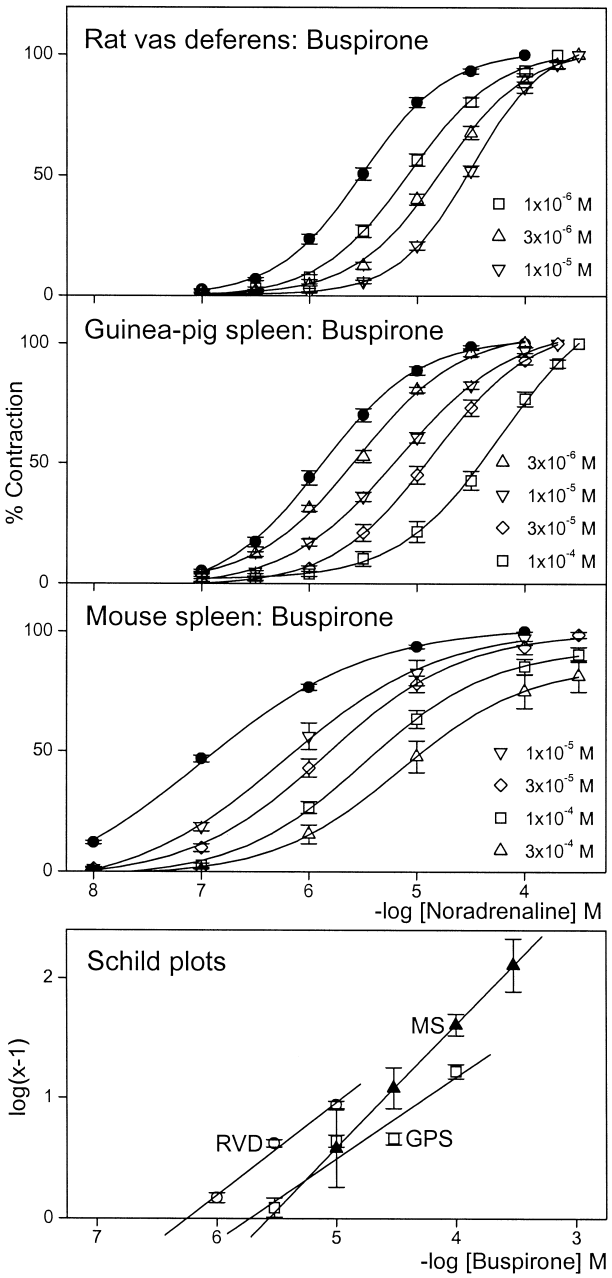

Fig. 2. Representative concentration–response curves for noradrenaline to evoke contraction of rat vas deferens, guinea-pig spleen and mouse spleen in the absence (filled circles) or presence of increasing concentrations of buspirone (open symbols) equilibrated with the tissue for 20–30 min (means ± S.E.M., $n = 12$–24 for the control, $n = 5$–12 in the presence of each concentration of buspirone). Bottom: Schild plots for the antagonism by buspirone of noradrenaline-induced contractions in rat vas deferens (RVD), guinea-pig spleen (GPS) and mouse spleen (MS). Given are means ± S.E.M. of $n = 18$ for RVD, $n = 4$–12 for GPS and $n = 4$–7 for MS.

In constant-pressure perfused rat kidney, buspirone (10^{-10}–10^{-6} mol), BMY 7378 (10^{-11}–10^{-7} mol) and

MDL 73005EF (10^{-10}–10^{-7} mol) dose dependently and reversibly increased perfusion flow when injected within 2 s into the renal inflow tract during vasoconstriction evoked by continuous noradrenaline perfusion (6×10^{-7} M). Neither BMY 7378 nor MDL 73005EF caused vasoconstriction or had an effect on basal perfusion flow in the absence of noradrenaline. Dose–response curves for the drugs to reverse the vasoconstriction caused by noradrenaline are depicted in Fig. 3 (top), and their $-\log ED_{50}$ values for a half-maximal vasodilator effect are listed in Table 1.

Without vasoconstriction by noradrenaline, however, bolus injections of buspirone (10^{-9}–10^{-6} mol) caused a weak decrease in basal perfusion flow of maximally 10.7 $\pm$ 1.4% at 10^{-6} mol (means $\pm$ S.E.M., $n = 6$) (Fig. 3, middle). The peak effect of vasoconstriction was achieved within 15 s after buspirone injection, remained stable for at least 1 min and then declined within 7–10 min. The effect of a maximally effective buspirone dose (10^{-6} mol) could be dose dependently and significantly attenuated by concomitant injection of low doses of the α_{1D}-adrenoceptor-selective antagonist BMY 7378 (threshold dose 3×10^{-9} mol), but hardly by 100-fold higher doses of the α_{1A}-adrenoceptor-selective antagonist B8805-033 (threshold dose 3×10^{-7} mol) (Fig. 3, bottom). These observations are consistent with the presence of constrictor α_{1D}-adrenoceptors in the rat renal vasculature which are susceptible to activation by buspirone.

A highly significant correlation resulted when the potencies of buspirone and the reference drugs to attenuate noradrenaline-induced renal vasocontriction due to α_{1A}-adrenoceptor blockade were compared to the affinity data from experiments for competitive α_{1A}-adrenoceptor antagonism in rat vas deferens ($r = 0.970$, $P < 0.001$; $\beta = 1.26$ not significantly different from 1.00, $P > 0.05$) (Fig. 4, top). In both preparations, tamsulosin, 5-methyl-urapidil and B8805-055 were the most potent antagonists of noradrenaline; however, the weakest antagonists, buspirone and MDL 73005EF, showed a slightly different rank order of affinity in rat vas deferens (buspirone > MDL 73005EF) compared to their potency in rat kidney (MDL 73005EF > buspirone). This might be due to concomitantly evoked vasoconstriction caused by stimulation of α_{1D}-adrenoceptors by buspirone in perfused rat kidney, which would functionally counteract the ability of buspirone to reverse noradrenaline-induced vasoconstriction by blockade of α_{1A}-adrenoceptors. This would make buspirone less effective as an antagonist in this tissue.

3.2. α_{1B}-adrenoceptors: guinea-pig and mouse splenic strips

In isolated splenic strips from guinea-pig and mouse, buspirone (3×10^{-6}–10^{-4} M in guinea-pig spleen; 10^{-5}–3×10^{-4} M in mouse spleen; Fig. 2, middle), BMY 7378 (10^{-6}–10^{-5} M in guinea-pig spleen; 3×10^{-7}–3×10^{-6} M in mouse spleen) and MDL 73005EF (10^{-6}–10^{-5} M in guinea-pig spleen; 3×10^{-7}–10^{-5} M in mouse spleen) caused parallel shifts to the right of the noradrenaline

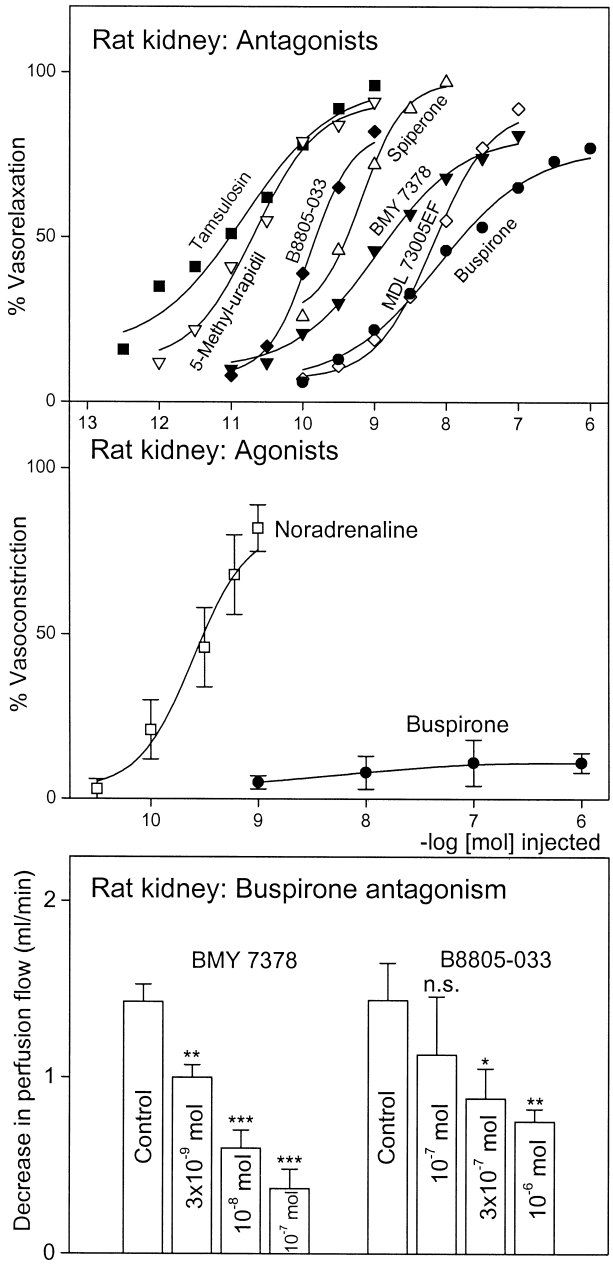

Fig. 3. Top: Dose–response curves for inhibition by buspirone and reference antagonists of renal vasoconstriction induced by the continuous presence of 6×10^{-7} M noradrenaline in perfused rat kidney (means without S.E.M. < 10% of $n = 6$–7). Middle: Dose–response curves for buspirone in comparison with noradrenaline to evoke renal vasoconstriction in perfused rat kidney at basal vascular tone (means $\pm$ S.D., $n = 6$–9). Bottom: Vasoconstrictor effect of buspirone (10^{-6} mol) in the absence (control columns: 27 values from nine kidneys prior to BMY 7378, 12 values from eight kidneys prior to B8805-033) and presence of BMY 7378 (3×10^{-9}, 10^{-8} and 10^{-7} mol; $n = 5$–9) and of B8805-033 (10^{-7}, 3×10^{-7} and 10^{-6} mol; $n = 6$) in perfused rat kidney (means $\pm$ S.E.M.). Significant differences between pre-drug control values for buspirone alone and those in the presence of the antagonists are indicated (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant, $P > 0.05$).

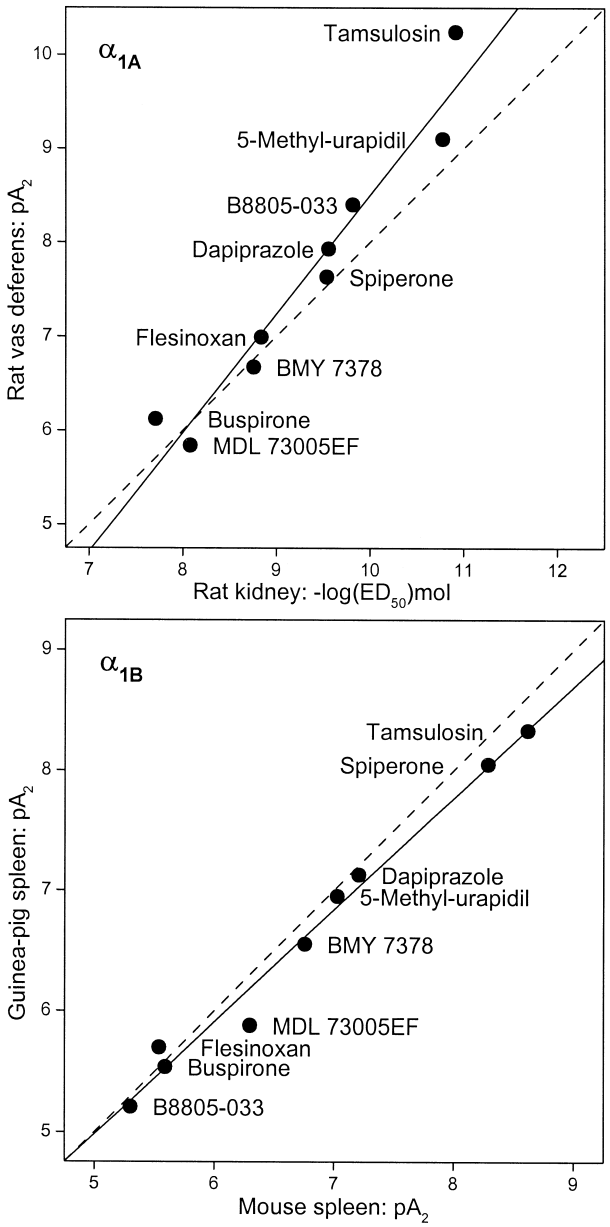

Fig. 4. Top: Relationship between the potency of antagonists to reverse noradrenaline-induced vasoconstriction in rat kidneys ($-\log ED_{50}$ values) and their affinity (pA_2 values) at α_{1A}-adrenoceptors in rat vas deferens. Bottom: Comparison of the affinity (pA_2 values) of buspirone and the reference antagonists listed in Table 1 at α_{1B}-adrenoceptors in guinea-pig and mouse spleen. As evidence for receptor identity ($pA_2 = pA_2$) in the two tissues, the normal regression line of the experimental data points (solid) did not significantly deviate from the depicted theoretical equality line (dashed).

concentration–response curves. None of the compounds in concentrations up to 10^{-4} M was agonistic in either tissue. The pA_2 values obtained from constrained Schild plots were 5.54 and 5.59 for buspirone (Fig. 2, bottom), 6.55 and 6.76 for BMY 7378, and 5.88 and 6.30 for MDL 73005EF at α_{1B}-adrenoceptors in guinea-pig and mouse spleen, respectively. The affinities of these compounds and those of the reference antagonists are listed in Table 1. In both tissues, the α_{1B}-adrenoceptor-selective antagonist,

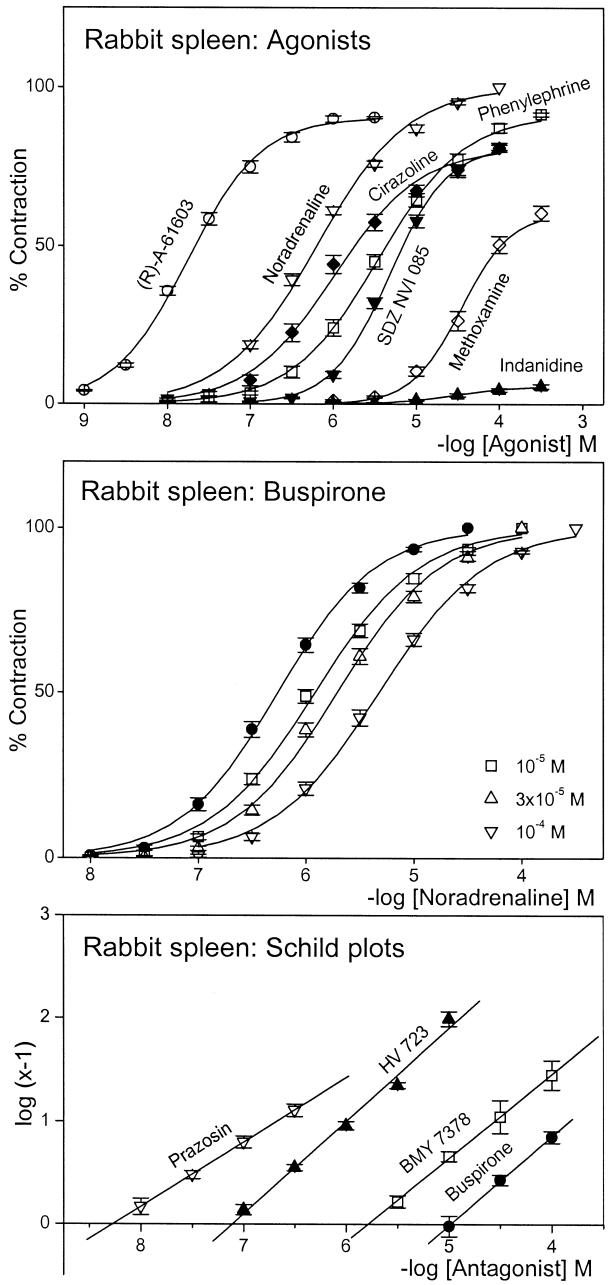

Fig. 5. Top: Concentration–response curves for α_1-adrenoceptor agonists, with responses expressed as a percentage of the maximum contraction elicited by noradrenaline (100%), in rabbit spleen (means ± S.E.M., n = 16–18). Middle: Representative concentration–response curves of noradrenaline to evoke contraction of rabbit spleen in the absence (filled circles) or presence of increasing concentrations of buspirone (open symbols) equilibrated with the tissue for 30 min (means ± S.E.M., $n = 18$ for the control, $n = 9$ in the presence of each concentration of buspirone). Bottom: Schild plots for the antagonism by buspirone and reference antagonists of contractions evoked by noradrenaline in rabbit spleen (means ± S.E.M., n = 9–12).

spiperone, was nearly as potent as tamsulosin, whereas the highly selective α_{1A}-adrenoceptor antagonist, B8805-033, displayed the weakest affinity at splenic α_{1B}-adrenoceptors. A highly significant correlation was obtained ($r = 0.992$, $P < 0.001$; $\beta = 0.93$ not significantly different from 1.00, $P > 0.05$) by comparing the two sets of antagonist affinities in these two tissues endowed with α_{1B}-adrenoceptors (Fig. 4, bottom).

3.3. Putative α_{1L}-adrenoceptors: rabbit spleen

Buspirone, up to 10^{-4} M, did not show any contractile effect in rabbit splenic strips. The agonists noradrenaline ($pD_2 = 6.29$, i.a = 1.00), (*R*)-A-61603 ($pD_2 = 7.75$, i.a. = 0.90 ± 0.04), cirazoline ($pD_2 = 6.06$, i.a. = 0.81 ± 0.05), phenylephrine ($pD_2 = 5.48$, i.a. = 0.91 ± 0.01), SDZ NVI 085 ($pD_2 = 5.35$; i.a. = 0.81 ± 0.04) and methoxamine ($pD_2 = 4.40$, i.a. = 0.60 ± 0.08; means ± S.E.M., $n = 16$–18) caused concentration-dependent contraction of the tissue, whereas indanidine was nearly inactive (i.a. = 0.06 ± 0.02) (Fig. 5, top). Buspirone (10^{-5}–10^{-4} M) caused competitive antagonism of noradrenaline-evoked splenic contractions, resulting in a pA_2 value of 4.99 and a relatively flat Schild plot with a slope of $\beta = 0.87$ (Fig. 5, middle and bottom). The affinities of the reference antagonists prazosin, HV 723 and BMY 7378 were 8.27 (at $\beta = 0.64$), 7.12 (at $\beta = 0.90$) and 5.81 (at $\beta = 0.81$), respectively (Fig. 5, bottom). The reason for the Schild plot slopes less than unity was not further investigated; however, the pA_2 values calculated for prazosin, HV 723 and BMY 7378 in rabbit spleen agreed well with their affinities at rabbit urethral α_{1L}-adrenoceptors ($pA_2 = 8.11$, 7.68 and 5.67, respectively; Leonardi et al., 1997; Testa et al., 1997), thereby excluding that α_{1D}-adrenoceptors participate in contraction of rabbit spleen.

3.4. α_{1D}-adrenoceptors: rat thoracic aorta and pulmonary artery

In rat thoracic aorta, BMY 7378 (10^{-8}–3×10^{-7} M) and MDL 73005EF (10^{-7}–3×10^{-6} M) clearly behaved as competitive antagonists against noradrenaline-evoked smooth muscle contraction ($pA_2 = 8.15$ and 7.23, respectively), whereas buspirone (10^{-8}–3×10^{-6} M) evoked contraction of the tissue ($pD_2 = 6.77 \pm 0.08$, i.a. = 0.40 ± 0.07) with a potency similar to that of the partial agonist

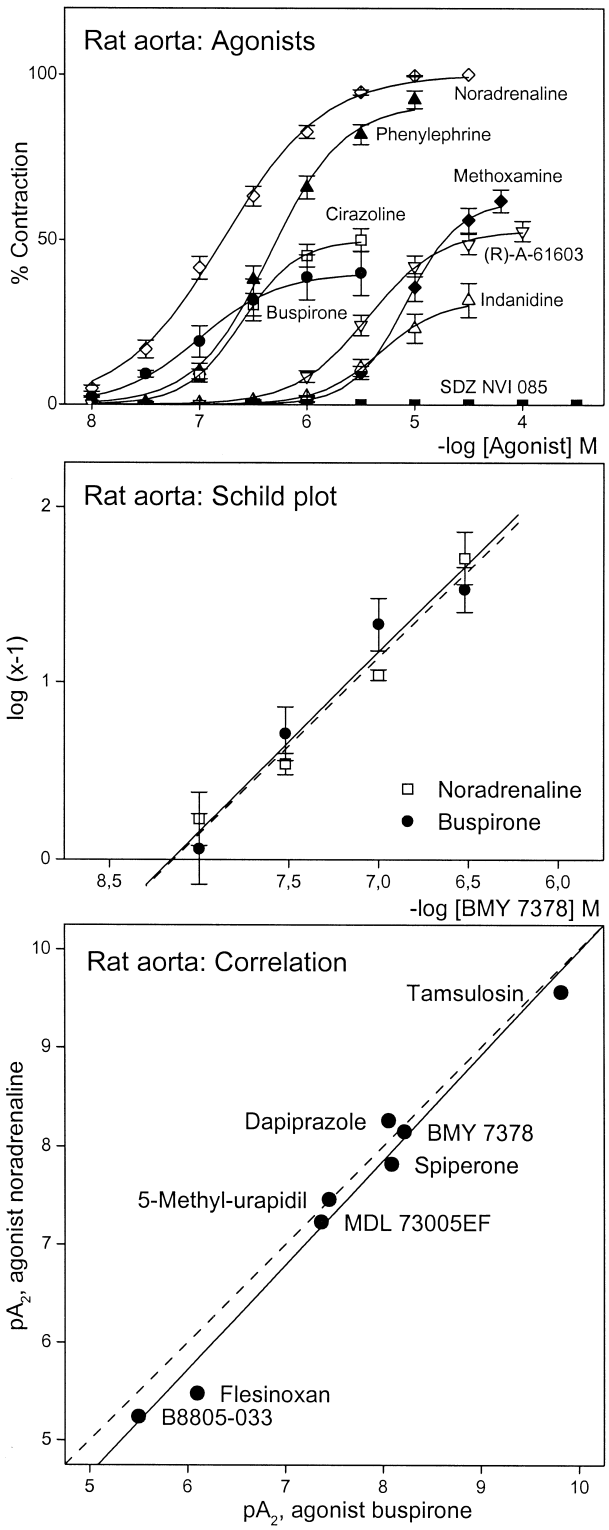

Fig. 6. Top: Concentration–response curves for the contractile effect of buspirone and reference α_1-adrenoceptor agonists, with responses expressed as a percentage of the maximum contraction elicited by noradrenaline (100%), in rat aorta (means ± S.E.M. $n = 17$–24). Middle: Schild plots for the antagonism by BMY 7378 of contractions evoked by noradrenaline (dashed line) and buspirone (solid line) in rat aorta (means ± S.E.M., $n = 3$–6 and 5–6, respectively). Bottom: Comparison of the affinities (pA_2 values) of the reference antagonists at α_{1D}-adrenoceptors in rat aorta with either noradrenaline or buspirone used as agonist. As evidence for receptor identity ($pA_2 = pA_2$) in the tissue activated by both agonists, the normal regression line of the experimental data points (solid) did not significantly deviate from the depicted theoretical equality line (dashed).

cirazoline ($pD_2 = 6.59 \pm 0.09$, i.a. 0.50 ± 0.16). In contrast to the full or nearly full agonists, noradrenaline ($pD_2 = 7.20 \pm 0.08$, i.a. = 1.00) and phenylephrine ($pD_2 = 6.37 \pm 0.06$, i.a. $= 0.91 \pm 0.11$), methoxamine ($pD_2 = 5.05 \pm 0.05$, i.a. $= 0.62 \pm 0.15$), indanidine ($pD_2 = 5.30 \pm 0.09$, i.a. $= 0.32 \pm 0.07$) and the selective α_{1A}-adrenoceptor agonist, (*R*)-A-61603 ($pD_2 = 5.45 \pm 0.07$, i.a. $= 0.53 \pm 0.03$; means ± S.E.M., $n = 17–24$) behaved as weak partial agonists, whereas SDZ NVI 085 was ineffective up to a concentration of 3×10^{-4} M (Fig. 6, top).

When buspirone ($10^{-8}–3 \times 10^{-6}$ M) was used to evoke stable and reproducible contractions in rat thoracic aorta, the affinities of the antagonists (pA_2 values) were nearly identical to those against noradrenaline (Table 1). Also the slopes of the Schild plots for all antagonists investigated against the agonist buspirone were not significantly different from 1.00 ($P > 0.05$), suggesting that buspirone activated only one α_1-adrenoceptor subtype in rat aorta. Representative Schild plots for the antagonism by BMY 7378 of contractions evoked by noradrenaline ($pA_2 = 8.15$; $\beta = 1.00$) and buspirone ($pA_2 = 8.22$; $\beta = 1.04$) are shown in Fig. 6 (middle). A highly significant correlation ($r = 0.991$, $P < 0.001$; $\beta = 1.18$ not significantly different from 1.00, $P > 0.05$) was obtained by comparing the two sets of antagonist affinities determined in noradrenaline- or buspirone-evoked rat aortic contraction experiments (Fig. 6, bottom). The antagonist affinities were independent of the agonists used, as would have been expected if the same α_{1D}-adrenoceptor was activated by both agonists in rat thoracic aorta.

Also in rat pulmonary artery, buspirone ($10^{-8}–3 \times 10^{-6}$ M) evoked sustained contraction. In most cases, reproducible responses were obtained during the third concentration–response curve. Its contractile potency, expressed as pD_2 value, was 7.16 ± 0.05 with a maximal effect expressed as i.a. $= 0.59 \pm 0.02$ relative to that of noradrenaline ($pD_2 = 7.42 \pm 0.01$, i.a. = 1.00). The values for other agonists investigated were as follows: phenylephrine ($pD_2 = 7.07 \pm 0.04$, i.a. $= 0.95 \pm 0.01$), cirazoline ($pD_2 = 7.16 \pm 0.02$, i.a. $= 0.56 \pm 0.03$), (*R*)-A-61603 ($pD_2 = 6.07 \pm 0.05$, i.a. $= 0.70 \pm 0.04$), methoxamine ($pD_2 = 5.49 \pm 0.02$, i.a. $= 0.79 \pm 0.03$) and indanidine ($pD_2 = 5.82 \pm 0.02$, i.a. $= 0.50 \pm 0.03$; means ± S.E.M., $n = 14–22$). SDZ NVI 085 was nearly inactive; its maximal effect, a 9% contraction (i.a. $= 0.09 \pm 0.04$), was reached at 3×10^{-5} M (Fig. 7, top).

The agonist curves for rat pulmonary artery resembled those for rat aorta. From their potencies (pD_2) and elicited maximal contractile effects (intrinsic activity) in both vascular preparations, phenylephrine was 3-fold weaker than noradrenaline and nearly equipotent to buspirone and cirazoline, although the latter two agonists evoked a contraction that was 60% of the maximal effect of noradrenaline. A third group of compounds consisted of the weaker partial agonists (*R*)-A-61603, methoxamine and indanidine, and the nearly ineffective SDZ NVI 085. Consistently 2- to 3-fold lower concentrations of buspirone and the reference agonists were necessary to evoke half-maximal contraction of rat pulmonary artery as compared to

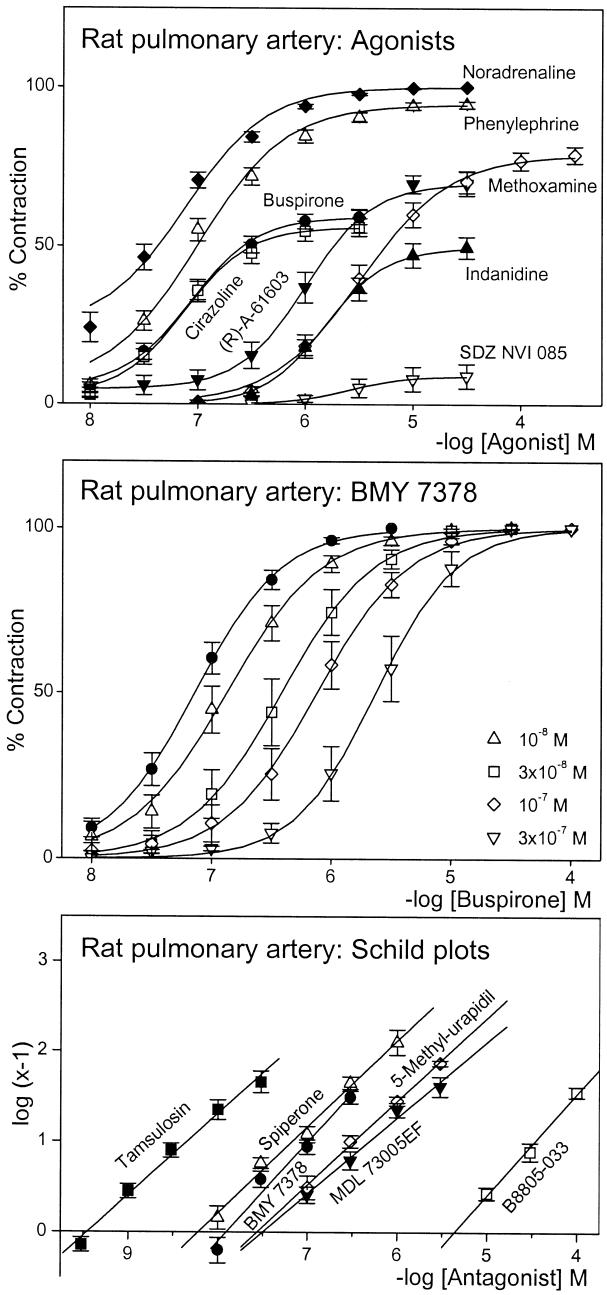

Fig. 7. Top: Concentration–response curves for the contractile effect of buspirone and reference α_1-adrenoceptor agonists, with responses expressed as a percentage of the maximum contraction elicited by noradrenaline (100%), in rat pulmonary artery (means ± S.E.M., $n = 17–24$). Middle: Representative concentration–response curves of buspirone to evoke contraction of rat pulmonary artery in the absence (filled circles) or presence of increasing concentrations of BMY 7378 (open symbols) equilibrated with the tissue for 30 min (means ± S.E.M. of $n = 14$ for the control and $n = 6–8$ in the presence of each concentration of BMY 7378). Bottom: Schild plots for the antagonism by a series of antagonists against buspirone-induced contractions in rat pulmonary artery (means ± S.E.M., $n = 6–9$).

those in rat aorta, possibly reflecting a higher receptor reserve in rat pulmonary artery than in aorta.

When buspirone was used as the agonist in rat pulmonary artery, the six antagonists listed in Table 2 caused parallel shifts to the right of the buspirone concentration–response curve without affecting its maximum contraction to any noticeable extent, indicating competitive antagonism at α_1-adrenoceptors in this tissue. As an example, the effect of BMY 7378 (10^{-8}–3×10^{-7} M) against buspirone-evoked contractions is depicted in Fig. 7 (middle). The regression lines for all antagonists investigated, i.e., BMY 7378, MDL 73005EF, spiperone, tamsulosin, 5-methyl-urapidil and B8805-033, were linear through the concentration range tested (Fig. 7, bottom) and had a slope not significantly different from unity ($P > 0.05$) (Table 2). Thus, no indication was obtained for the presence of multiple subtypes of α_1-adrenoceptors activated by buspirone in this tissue.

An excellent correlation was found ($r = 989$, $P < 0.001$; $\beta = 0.96$ not significantly different from 1.00, $P > 0.05$; Fig. 8, top) when we compared five antagonist affinities (Table 1) determined in rat aorta experiments using buspirone as agonist with their average pK_i values for cloned α_{1d}-adrenoceptors taken from the literature (Table 2). However, no significant correlations were obtained when these functional affinities were compared to values for cloned α_{1a}-adrenoceptors ($r = 0.702$, $P > 0.05$; $\beta = 1.28$ significantly different from 1.00, $P < 0.05$) and for cloned α_{1b}-adrenoceptors ($r = 0.771$, $P > 0.05$; $\beta = 0.98$ not significantly different from 1.00, $P < 0.01$). Likewise, the functional affinities of these five subtype-discriminating antagonists determined against buspirone in rat pulmonary artery (Table 2) correlated well with average pK_i values for cloned α_{1d}-adrenoceptors ($r = 0.968$, $P < 0.01$; $\beta = 1.16$ not significantly different from 1.00, $P > 0.05$), but not with α_{1a}-adrenoceptors ($r = 0.734$, $P > 0.05$; $\beta = 1.67$ significantly different from 1.00, $P < 0.001$) and α_{1b}-adrenoceptors ($r = 0.850$, $P > 0.05$; $\beta = 1.33$ significantly different from 1.00, $P < 0.01$; Fig. 8, bottom). Thus the α_1-adrenoceptor subtype activated by buspirone in rat aorta and pulmonary artery is pharmacologically equivalent to the cloned and expressed α_{1d}-adrenoceptor.

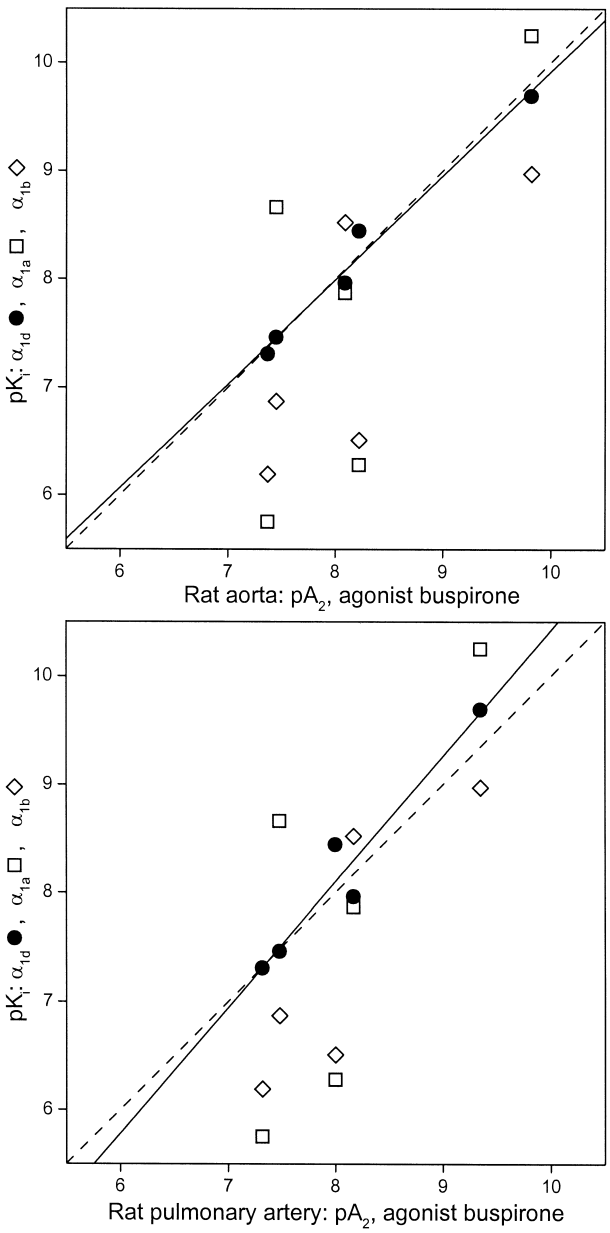

Fig. 8. Relationship between the affinities of the antagonists (pA_2) listed in Table 1Table 2 (tamsulosin, BMY 7378, spiperone, 5-methyl-urapidil and MDL 73005F) determined against the agonist buspirone in rat aorta (top) and rat pulmonary artery (bottom) and their binding affinities (pK_i) at cloned α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors (taken from the literature and listed in Table 2). For receptor identity ($pA_2 = pK_i$), the normal regression line (solid) of the experimental data points for affinity at α_{1d}-adrenoceptors (solid circles) should not deviate significantly from the depicted theoretical equality line (dashed). The regression lines of drugs with affinity for cloned α_{1a}- (open squares) and α_{1b}-adrenoceptors (open diamonds) are not depicted.

4. Discussion

4.1. General considerations

The pharmacological profile of the anxiolytic drug, buspirone, in addition to its nanomolar affinity for 5-HT_{1A} receptors (Goa and Ward, 1986), includes interaction with α_1-adrenoceptors at micromolar concentrations. Buspirone has been reported to have a 3- and 10-fold higher binding affinity at the cloned animal α_{1d}-adrenoceptor than at the α_{1a}- and α_{1b}-adrenoceptors, respectively (Saussy et al., 1996); however, since no data on its functional affinity at these receptors are known, we investigated these properties by using a number of isolated tissues endowed with the various subtypes, such as the rat vas deferens and perfused rat kidney which constitute reliable functional assay mate-

rial for investigating the A subtype of α_1-adrenoceptors (Eltze and Boer, 1992). The affinity characteristics of antagonists that inhibit contraction in guinea-pig and mouse spleen are identical to those at cloned α_{1b}-adrenoceptors (Eltze, 1994, 1996). The rat aorta can now be regarded as being predominantly supplied with α_{1D}-adrenoceptors mediating contraction to agonists (Saussy et al., 1994; Kenny et al., 1995; Testa et al., 1995). The contractions elicited by phenylephrine of the rat pulmonary artery have been identified to be mediated in part via the α_{1D}-subtype of adrenoceptors (Hussain and Marshall, 1997), and, in addition to α_{1A}-adrenoceptors in rat kidney, α_{1D}-adrenoceptors have been detected in rat renal artery (Piascik et al., 1994; Villalobos-Molina et al., 1997).

4.2. Antagonist activity of buspirone

These reliable functional methods confirmed BMY 7378 and MDL 73005EF to be selective (30- and 20-fold, respectively) for α_{1D}-adrenoceptors rather than for α_{1A}- and α_{1B}-adrenoceptors, but in contrast to previously reported data from binding experiments with human (selectivity for BMY 7378 and MDL 73005EF: d > b > a) and animal cloned α_1-adrenoceptor subtypes (selectivity for BMY 7378: d > a > b; for MDL 73005EF: d > b > a) (Saussy et al., 1996), neither compound greatly discriminated between the A and B subtypes in our functional experiments ($\alpha_{1D} > \alpha_{1A} = \alpha_{1B}$).

Buspirone was a competitive antagonist without any observable agonist activity at α_{1A}-adrenoceptors in rat vas deferens and at α_{1B}-adrenoceptors in guinea-pig and mouse spleen. In these functional assays, buspirone displayed an approximately 3-fold higher affinity at α_{1A}-adrenoceptors (rat vas deferens: $pA_2 = 6.12$) than at α_{1B}-adrenoceptors (guinea-pig and mouse spleen: $pA_2 = 5.54$ and 5.59, respectively), which is consistent with previous data derived from binding experiments, demonstrating an average 3-fold higher affinity at cloned α_{1a}-adrenoceptors (rat and human: $pK_i = 5.66$ and 5.92, respectively) than at α_{1b}-adrenoceptors (hamster and human: $pK_i = 5.11$ and 5.29, respectively)(Saussy et al., 1996).

Buspirone behaved as a weak competitive antagonist in rabbit spleen ($pA_2 = 4.99$), and contraction was elicited by stimulation of an α_1-adrenoceptor with characteristics similar to those of the putative L subtype (Oriowo, 1998). The L subtype has previously been detected in some blood vessels and is defined by its relatively low affinity for both prazosin (< 9.0) and HV 723 (< 8.0)(Muramatsu et al., 1990b). Our pA_2 values for prazosin, HV 723 and BMY 7378 (8.27, 7.12 and 5.81, respectively) determined in rabbit spleen are in agreement with those reported for the drugs at α_{1L}-adrenoceptors in rabbit urethra (8.11, 7.67 and 5.67, respectively; Leonardi et al., 1997; Testa et al., 1997), and also with those found for prazosin and BMY 7378 at α_{1L}-adrenoceptors in rabbit corpus cavernosum (8.04 and 5.89, respectively; Chalmers et al., 1997), and thus confirm the recent suggestion of Oriowo (1998) of a putative α_{1L}-adrenoceptor mediating contraction of rabbit spleen in response to phenylephrine and (*R*)-A-61603. Furthermore, the low affinity of BMY 7378 ($pA_2 = 5.81$) obtained in our study also excludes that α_{1D}-adrenoceptors participate in contraction of rabbit spleen.

4.3. Agonist activity of buspirone

When the potencies of buspirone and the reference antagonists to attenuate noradrenaline-induced renal vasoconstriction were plotted against their affinity values derived from contraction experiments in rat vas deferens, a highly significant correlation resulted, suggesting that buspirone blocks α_{1A}-adrenoceptors in the rat renal vasculature with a comparable potency as in rat vas deferens when noradrenaline is used as agonist in both tissues. However, since the presence of mRNA for the α_{1d}-adrenoceptor subtype (Piascik et al., 1994) and its additional participation in vasoconstriction in response to phenylephrine (Villalobos-Molina et al., 1997) have been reported in the rat renal artery, perfusion of the isolated rat kidney via the abdominal aorta would allow both intrarenal resistance vessels containing mainly α_{1A}-adrenoceptors (Elhawary et al., 1992) and extrarenal vascular beds, like the renal artery, that are apparently endowed with both α_{1A}- and α_{1D}-adrenoceptors (Han et al., 1990; Villalobos-Molina et al., 1997) to respond to α_1-adrenoceptor agonists like noradrenaline and phenylephrine. Thus the weak vasoconstriction elicited by buspirone under conditions with no additional vasoconstrictor, can be explained by activation of α_{1D}-adrenoceptors, since its maximal response to an injected high dose of 10^{-6} mol was significantly attenuated by low doses (threshold 3×10^{-9} mol) of the selective α_{1D}-adrenoceptor antagonist BMY 7378, but only by high doses (threshold 3×10^{-7} mol) of the highly selective α_{1A}-adrenoceptor antagonist B8805-033. Based on their ability to cause a 50% inhibition of buspirone-evoked vasoconstriction, BMY 7378 ($ED_{50} = 5 \times 10^{-9}$ mol) proved to be 200-fold more potent than B8805-033 ($ED_{50} = 10^{-6}$ mol), their difference in potency being nearly as great as that in affinity observed at α_{1D}-adrenoceptors in rat aorta (factor 520) and rat pulmonary artery (factor 350) when buspirone was used as the agonist. In contrast, the affinity difference between BMY 7378 and B8805-033 was weaker at subtype B (factor 20–30) and inverse at subtype A (factor 0.02–0.1), thus making these α_1-adrenoceptor subtypes most unlikely to participate in the response elicited by buspirone in rat kidney. The site at which buspirone acts to cause vasoconstriction cannot be deduced from our perfusion experiments, but may be possibly located at the level of the renal artery where the subtype D has been demonstrated to exist (Villalobos-Molina et al., 1997). Consistent with these findings are the

results from our preliminary experiments on rat isolated renal artery, which revealed that buspirone (10^{-8}–3×10^{-6} M) evoked a contraction of the vessel that was between 20 and 30% of maximum (defined by 3×10^{-5} M noradrenaline). The contraction could be competitively antagonized by BMY 7378, MDL 73005EF and B8805-033 with pA_2 values of 8.3, 7.6 and 5.6, respectively (not shown), values that are typically found for the antagonists in α_{1D}-adrenoceptor tissues like rat aorta and pulmonary artery. This suggests that the site of the vasoconstriction observed after buspirone in the perfused rat kidney may be indeed the renal artery.

In rat aorta, buspirone behaved as a partial agonist of similar potency and intrinsic activity as the α_1-adrenoceptor agonist cirazoline. It is now generally accepted that contractile responses of this tissue to α_1-adrenoceptor agonists like noradrenaline and phenylephrine are mediated via activation of the D subtype (Saussy et al., 1994; Kenny et al., 1995; Testa et al., 1995; Eltze, 1996). To test the hypothesis that buspirone also activates α_{1D}-adrenoceptors and not 5-HT_{1A} receptors in the rat aorta, a series of selective α_1-adrenoceptor antagonists were evaluated for inhibition of buspirone-evoked contractions. However, despite the different antagonist selectivity for α_1-adrenoceptor subtypes, the slopes of the Schild plots for all of the compounds investigated were not significantly different from unity. This is consistent with a single α_1-adrenoceptor subtype mediating contraction in response to buspirone. The contraction was antagonized by these compounds with affinity values identical to those determined against noradrenaline. This suggests that aortic contractions elicited by both agonists are mediated by the same α_1-adrenoceptor, i.e., the D subtype, which exhibits a pharmacological equivalency to cloned and expressed α_{1d}-adrenoceptors.

In the other vascular preparation, the rat pulmonary artery, buspirone was an agonist of similar potency and intrinsic activity as in the rat aorta. Also the subtype-discriminating antagonists, BMY 7378, MDL 73005EF, 5-methyl-urapidil, B8805-033, tamsulosin and spiperone, were competitive against buspirone-evoked contractions with Schild plot slopes of unity and affinity values that were consistent with those assessed at α_{1D}-adrenoceptors in rat aorta by using either noradrenaline or buspirone as agonist, and aligned exactly with those measured for cloned α_{1d}-adrenoceptors. Previous results obtained with the agonist phenylephrine in rat pulmonary artery yielded a non-competitive inhibition by BMY 7378 and a discontinuity of the points in the biphasic Schild plot, resulting in a relatively high affinity constant ($pA_2 = 8.9$) for the antagonist (Hussain and Marshall, 1997). The reason for the deviation from competitiveness between both drugs is unclear but suggests that there is α_1-adrenoceptor heterogeneity in the rat pulmonary artery, the contraction of which, e.g., by phenylephrine, is only in part mediated by the D subtype. In the present study, however, the interaction between BMY 7378 and the agonist buspirone was clearly competitive in nature with a Schild plot slope of unity, thereby excluding the involvement of more than one receptor in the contractile response elicited by buspirone. The affinity value obtained for BMY 7378 ($pA_2 = 8.00$) agrees with those functionally determined for the antagonist in rat aorta using noradrenaline or buspirone as agonist ($pA_2 = 8.15$ and 8.22, respectively) and also with its average affinity at cloned α_{1d}-adrenoceptors ($pK_i = 8.44$). A convincing conformity of affinity data obtained in these three functional assays with those derived in binding experiments with cloned α_{1d}-adrenoceptors was also found for MDL 73005EF ($pA_2 = 7.32$, 7.23 and 7.37, respectively; $pK_i = 7.31$) and 5-methyl-urapidil ($pA_2 = 7.48$, 7.46 and 7.45, respectively; $pK_i = 7.46$). As further evidence, the affinity of the 1000-fold selective α_{1A}-adrenoceptor antagonist, B8805-033, remained low, as expected, in rat pulmonary artery ($pA_2 = 5.48$) and is consistent with values in rat aorta ($pA_2 = 5.24$ and 5.50). Thus, whatever the subtype(s) in rat pulmonary artery may be and its contribution to the phenylephrine-induced contraction (Hussain and Marshall, 1997), the use of the agonist buspirone and the competitive inhibition of its response by antagonists with varying selectivity for the subtypes A, B and D clearly indicate that buspirone activates a single α_1-adrenoceptor, namely the D subtype, in this tissue.

4.4. Effects of buspirone in other preparations endowed with different α_1-adrenoceptor subtypes

In rat vas deferens, a tissue with a functional α_{1A}-adrenoceptor supply, and also in guinea-pig and mouse spleen, which are endowed with α_{1B}-adrenoceptors, buspirone displayed no agonist activity. Not surprisingly and now explicable, this is consistent with the previous observation that buspirone did not elicit a contractile response in guinea-pig aorta (Rimele et al., 1987), a preparation known to contain functional α_{1A}-adrenoceptors (Oriowo, 1994). No vasoconstriction by buspirone was observed in dog femoral artery (Rimele et al., 1987), the postjunctional α_1-adrenoceptor subtype of which resembles the A subtype, based on the potency order of a series of agonists like adrenaline, noradrenaline, phenylephrine and methoxamine to evoke contraction (Sinanovic and Chiba, 1987) and the high potency of 5-methyl-urapidil against noradrenaline-evoked contractions ($pA_2 = 8.43$; Kohno et al., 1994). Also in dog saphenous vein, which has been characterized to be endowed with a mixed population of α_{1A}- and α_{1B}-adrenoceptors activated by noradrenaline and phenylephrine (Muramatsu et al., 1990b; Hicks et al., 1991), buspirone evoked no contraction (Rimele et al., 1987).

Buspirone has repeatedly been shown to be capable of contracting the rabbit thoracic aorta (Rimele et al., 1987; Gürdal et al., 1992; Castillo et al., 1993). Vargas and Gorman (1995) have summarized the results of various investigators suggesting that this tissue is functionally

supplied with α_{1A}-, α_{1B}- and α_{1L}-adrenoceptor subtypes mediating the contractile response to noradrenaline and methoxamine (see Section 1, Table 3). Particularly, after treatment with chloroethylclonidine, the contractile response to noradrenaline and its inhibition by antagonists at remaining α_1-adrenoceptors in rabbit aorta show similarities to those elicited by stimulation of the putative L subtype typically detected in tissues of the lower urinary tract (Testa et al., 1997). However, the specific contribution of each of the at least three defined subtypes mediating contraction of rabbit aorta to the response to one particular agonist appears not to be well understood (Muramatsu et al., 1998). Furthermore, several factors, including receptor reserve and G protein composition, might influence α-adrenoceptor agonist-mediated responses in different tissues (Bevan et al., 1988; Minneman, 1988). However, the good correlation obtained between pK_b values of a series of antagonists against noradrenaline-induced contraction of rabbit aorta and their pK_i for inhibition of [^{3}H]prazosin binding at human recombinant α_{1d}-adrenoceptors led Leonardi et al. (1997) to suggest that α_{1D}-adrenoceptors might also be involved in contraction of the rabbit aorta. A similar conclusion on the possible existence of α_{1D}-adrenoceptors in this tissue has been recently reached by Satoh et al. (1998). The potency of 5-methyl-urapidil to competitively antagonize buspirone-evoked contractions (approximately calculated $pA_2 = 7.5–7.8$) and their sensitivity to blockade by chloroethylclonidine in rabbit aorta (Castillo et al., 1993) is consistent with the characteristics of drugs that act at the α_{1D}-adrenoceptors typically found in the rat aorta (5-methyl-urapidil, $pA_2 = 7.45$, this paper; sensitivity to chloroethylclonidine, Eltze and Boer, 1992), and with the binding affinity of 5-methyl-urapidil at cloned α_{1d}-adrenoceptors (average $pK_i = 7.46$; Table 2). Thus it is feasible that the contraction elicited by buspirone observed in previous studies in rabbit aorta (Rimele et al., 1987; Gürdal et al., 1992; Castillo et al., 1993) is mediated by a small portion of co-existing α_{1D}-adrenoceptors stimulated by buspirone. Consistent with this suggestion is the recent detection of abundant mRNA for the α_{1d}-adrenoceptor in this tissue (Suzuki et al., 1997). Preliminary experiments in our labo-

Table 3
Summary of effects observed for buspirone in this study and those taken from the literature for isolated tissues endowed with different α_1-adrenoceptor subtypes A, B, D and L, characterized by means of functional experiments. The preliminary results on the contractile effect of buspirone and its inhibition by antagonists in rabbit aorta and rat renal artery mentioned in Section 4 are also included

Tissue	Effect	Subtype	References
Rat aorta	Contraction	–	Rimele et al. (1987); Saussy et al. (1996)
	Contraction	D	This study
	–	D	Saussy et al. (1994); Kenny et al. (1995); Testa et al. (1995); Eltze (1996)
Rat pulm. artery	Contraction	D	This study
	–	D	Hussain and Marshall (1997)
Rat kidney	Constriction	D	This study
	–	A	Eltze et al. (1991); Elhawary et al. (1992); Blue et al. (1995)
Rat renal artery	–	A	Han et al. (1990)
		D, A	Piascik et al. (1994); Villalobos-Molina et al. (1997)
	Contraction	D	This study (preliminary results: BMY 7378 $pA_2 = 8.3$, MDL 73005EF $pA_2 = 7.6$, B8805-033 $pA_2 = 5.6$)
Rabbit aorta	Contraction	–	Rimele et al. (1987); Gürdal et al. (1992)
	Contraction	A, B	Castillo et al. (1993)
	–	L, B	Muramatsu et al. (1990a, 1998)
	–	A, B	Suzuki et al. (1990); Oriowo and Ruffolo (1992)
	–	L	Testa et al. (1997)
	–	D	Leonardi et al. (1997)
	Contraction	D	This study (preliminary results: tamsulosin $pA_2 = 9.4$, BMY 7378 $pA_2 = 7.5$, MDL 73005EF $pA_2 = 6.6$, B8805-033 $pA_2 = 5.6$)
Rat vas deferens	No contraction	A	This study
	–	A	Han et al. (1987); Eltze and Boer (1992); Kenny et al. (1994)
Guinea-pig spleen	No contraction	B	This study
	–	B	Eltze (1994)
Mouse spleen	No contraction	B	This study
	–	B	Eltze (1996)
Rabbit spleen	No contraction	L	This study
	–	L	Oriowo (1998)
Guinea-pig aorta	No contraction	–	Rimele et al. (1987)
	–	A	Oriowo (1994)
Dog femoral artery	No contraction	–	Rimele et al. (1987)
	–	A?	Sinanovic and Chiba (1987); Kohno et al. (1994)
Dog saph. vein	No contraction	–	Rimele et al. (1987)
	–	A, B	Muramatsu et al. (1990b); Hicks et al. (1991)

ratory revealed that tamsulosin, BMY 7378, MDL 73005EF and B8805-033 competitively antagonize buspirone-evoked contractions in rabbit aorta with pA_2 values of 9.4, 7.5, 6.6 and 5.6, respectively, which makes the subtype D, rather than the A, B or L subtypes, a likely candidate to be involved in such contraction (not shown).

The effects of buspirone described in detail in the present study and also our preliminary results mentioned in Section 4 as well as data taken from the literature are summarized in Table 3. In all tissues so far investigated and unequivocally (rat aorta, pulmonary artery, kidney, renal artery) or at least putatively endowed with α_{1D}-adrenoceptors (rabbit aorta), buspirone causes contraction, whereas in all tissues containing the subtype A (rat vas deferens, guinea-pig aorta, dog femoral artery), subtype B (guinea-pig and mouse spleen) or both (dog saphenous vein), and subtype L (rabbit spleen) buspirone does not cause contractile responses.

4.5. Conclusions

In conclusion, in functional studies of tissues containing the A subtype (rat vas deferens and perfused kidney), the B subtype (guinea-pig and mouse spleen) and the L subtype of α_1-adrenoceptors (rabbit spleen) buspirone is a weak antagonist, but behaves as a partial agonist of similar potency and intrinsic activity as cirazoline in two tissues bearing the D subtype of α_1-adrenoceptors (rat aorta and pulmonary artery), and as a weak partial agonist at additional constrictor α_{1D}-adrenoceptors in the rat renal vasculature. The potencies of antagonists to inhibit the effects of buspirone in rat aorta, pulmonary artery and perfused kidney are consistent with the responses being mediated via the α_{1D}-adrenoceptor, which is characterized by its high affinity for the selective α_{1D}-adrenoceptor antagonist, BMY 7378, and by its extremely low affinity for the selective α_{1A}-adrenoceptor antagonist, B8805-033. The affinity characteristics of a series of subtype-discriminating antagonists for α_{1D}-adrenoceptors in the rat aorta and pulmonary artery activated by buspirone exhibit a pharmacological equivalency to those of cloned and expressed α_{1d}-adrenoceptors. Thus, if the characteristics of buspirone are not only specific for the tissues used in this study, each being differently supplied with one or more of the four α_1-adrenoceptor subtypes A, B, D and L, but also represent a general phenomenon to be extended to other preparations, this drug may be a potentially valuable research tool for use in functional studies to assess the specific contribution of the α_{1D}-adrenoceptor to vascular or non-vascular smooth muscle contraction.

References

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Bevan, J.A., Bevan, R.D., Kite, K., Oriowo, M.A., 1988. Species differences in sensitivity of aortae to norepinephrine are related to α-adrenoceptor affinity. Trends Pharmacol. Sci. 9, 87–89.

Blue, D.R. Jr, Bonhaus, D.W., Ford, A.P.D.W., Pfister, J.R., Sharif, N.A., Shieh, I.A., Vimont, R.L., Williams, T.J., Clarke, D.E., 1995. Functional evidence equating the pharmacologically defined α_{1A}- and cloned α_{1C}-adrenoceptor: studies in the isolated perfused kidney of rat. Br. J. Pharmacol. 115, 283–294.

Castillo, C., Ibarra, M., Márquez, J.A., Villalobos-Molina, R., Hong, E., 1993. Pharmacological evidence for interactions between 5-HT_{1A} receptor agonists and subtypes of α_1-adrenoceptors on rabbit aorta. Eur. J. Pharmacol. 241, 141–148.

Castillo, C., Bobadilla, R.A., Ibarra, M., Castillo, E.F., Hong, E., 1995. A comparative study of the effects of some 5-HT_{1A} receptor agonists on the blood pressure of pithed rats. Arch. Med. Res. 26, 251–255.

Chalmers, D.H., Miller, A.M., Kenny, B.A., 1997. Characterization of the α_1-mediated responses of isolated rabbit corpus cavernosum. Br. J. Pharmacol. 120, 210P, Suppl.

Elhawary, A.D., Pettinger, W.A., Wolff, D.W., 1992. Subtype-selective alpha-1 adrenoceptor alkylation in the rat kidney and its effect on the vascular pressor response. J. Pharmacol. Exp. Ther. 260, 709–713.

Eltze, M., 1994. Functional characterization of the α_1-adrenoceptor subtype mediating contraction of the guinea-pig spleen. Eur. J. Pharmacol. 260, 211–220.

Eltze, M., 1996. Functional evidence for an α_{1B}-adrenoceptor mediating contraction of the mouse spleen. Eur. J. Pharmacol. 311, 187–198.

Eltze, M., 1997. Affinity of the miotic drug, dapiprazole, at α_1-adrenoceptor subtypes A, B and D. J. Pharm. Pharmacol. 49, 1091–1095.

Eltze, M., Boer, R., 1992. The adrenoceptor agonist, SDZ NVI 085, discriminates between α_{1A}- and α_{1B}-adrenoceptor subtypes in vas deferens, kidney and aorta of the rat. Eur. J. Pharmacol. 224, 125–136.

Eltze, M., Boer, R., Sanders, K.H., Kolassa, N., 1991. Vasodilatation elicited by 5-HT_{1A} receptor agonists in constant-pressure perfused rat kidney is mediated by blockade of α_{1A}-adrenoceptors. Eur. J. Pharmacol. 202, 33–44.

Eltze, M., Boer, R., Sanders, K.H., Prüsse, W., Ulrich, W.-R., 1996. B8805-033: an extremely α_{1A}-adrenoceptor-selective antagonist. Naunyn-Schmiedeberg's Arch. Pharmacol. 354, R9, Suppl.

Forray, C., Bard, J.A., Wetzel, J.M., Chiu, G., Shapiro, E., Tang, R., Lepor, H., Hartig, P.R., Weinshank, R.L., Branchek, T.A., Gluchowski, C., 1994. The α_1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned α_{1c} subtype. Mol. Pharmacol. 45, 703–708.

Goa, K.L., Ward, A., 1986. Buspirone: a preliminary review of its pharmacological properties and therapeutic efficacy as an anxiolytic. Drugs 32, 114–129.

Goetz, A.S., King, H.K., Ward, S.D.C., True, T.A., Rimele, T.J., Saussy, D.L., 1995. BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptors. Eur. J. Pharmacol. 272, R5.

Gross, G., Hanft, G., Rugevics, C., 1988. 5-Methylurapidil discriminates between subtypes of the α_1-adrenoceptor. Eur. J. Pharmacol. 151, 333–335.

Gürdal, H., Onaran, H.O., Tulunay, F.C., 1992. Role of α-adrenoceptors in the effects of buspirone and 5-carboxamidotryptamine in rabbit isolated thoracic aorta. Gen. Pharmacol. 23, 43–47.

Han, C.H., Abel, P.W., Minneman, K.P., 1987. α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature (London) 329, 333–335.

Han, C., Li, J., Minneman, K.P., 1990. Subtypes of α_1-adrenoceptors in blood vessels. Eur. J. Pharmacol. 190, 97–104.

Hanson, R.C., Braselton, J.P., Hayes, D.C., Snyder, R.W., White, J.B., Deitchman, D., 1986. Cardiovascular and renal effects of buspirone in several animal models. Gen. Pharmacol. 17, 267–274.

Hicks, P.E., Barras, M., Herman, G., Mauduit, P., Armstrong, J.M., Rossignol, B., 1991. α-Adrenoceptor subtypes in dog saphenous vein that mediate contraction and inositol phosphate production. Br. J. Pharmacol. 102, 151–161.

Hieble, J.P., Bylund, D.B., Clarke, D.E., Eickenburg, D.C., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Ruffolo, R.R., 1995. International Union of Pharmacology: X. Recommendations for nomenclature of α_1-adrenoceptors. Pharmacol. Rev. 47, 267–270.

Hussain, M.B., Marshall, I., 1997. Characterization of α_1-adrenoceptor subtypes mediating contractions to phenylephrine in rat thoracic aorta, mesenteric artery and pulmonary artery. Br. J. Pharmacol. 122, 849–858.

Kenny, B.A., Naylor, A.M., Greengrass, P.M., Russell, M.J., Friend, S.J., Read, A.M., Wyllie, M.G., 1994. Pharmacological properties of the cloned $\alpha_{1A/D}$-adrenoceptor subtype are consistent with the α_{1A}-adrenoceptor characterized in rat cerebral cortex and vas deferens. Br. J. Pharmacol. 111, 1003–1008.

Kenny, B.A., Chalmers, D.H., Philpot, P.C., Naylor, A.M., 1995. Characterization of a α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br. J. Pharmacol. 115, 981–986.

Kohno, Y., Saito, H., Takita, M., Kigoshi, S., Muramutsu, I., 1994. Heterogeneity of α_1-adrenoceptor subtypes involved in adrenergic contraction of dog blood vessels. Br. J. Pharmacol. 112, 1167–1173.

Leonardi, A., Hieble, J.P., Guarneri, L., Naselsky, D.P., Poggesi, E., Sironi, G., Sulpizio, A.C., Testa, R., 1997. Pharmacological characterization of the uroselective alpha-1 antagonist Rec 15/2739 (SB 216469): role of the alpha-1L adrenoceptor in tissue selectivity. Part I. J. Pharmacol. Exp. Ther. 281, 1272–1283.

Michel, A.D., Loury, D.N., Whiting, R.L., 1989. Identification of a single α_1-adrenoceptor corresponding to the α_1-subtype in rat submaxillary gland. Br. J. Pharmacol. 98, 883–889.

Minneman, K.P., 1988. α_1-Adrenergic receptor subtypes, inositol phosphates, and sources of cell Ca^{2+}. Pharmacol. Rev. 40, 87–119.

Minneman, K.P., Esbenshade, T.T., 1994. α_1-Adrenergic receptor subtypes. Annu. Rev. Pharmacol. Toxicol. 34, 117–133.

Muramutsu, I., Kogishi, S., Oshita, M., 1990a. Two distinct α_1-adrenoceptor subtypes involved in noradrenaline contraction of the rabbit thoracic aorta. Br. J. Pharmacol. 101, 662–666.

Muramatsu, I., Ohmura, T., Kigoshi, S., Hashimoto, S., Oshita, M., 1990b. Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. Br. J. Pharmacol. 99, 197–201.

Muramatsu, I., Murata, S., Isaka, M., Piao, H.L., Zhu, J., Suzuki, F., Miyamoto, S., Oshita, M., Watanabe, Y., Taniguchi, T., 1998. Alpha$_1$-adrenoceptor subtypes and two receptor systems in vascular tissues. Life Sci. 62, 1461–1465.

Oriowo, M.A., 1994. Alpha$_1$-adrenoceptor subtype(s) mediating noradrenaline-induced contractions of guinea-pig aorta. Fundam. Clin. Pharmacol. 8, 214–219.

Oriowo, M.A., 1998. Functional characterization of α_1-adrenoceptor subtypes in the rabbit spleen. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 301–307.

Oriowo, M.A., Ruffolo, R.R., 1992. Heterogeneity of postjunctional alpha$_1$-adrenoceptors in mammalian aortae: subclassification based on chloroethylclonidine, WB 4101 and nifedipine. J. Vasc. Res. 29, 33–40.

Perez, D.M., Piascik, M.T., Malik, M., Gaivin, R., Graham, R.M., 1994. Cloning, expression, and tissue distribution of the rat homolog of the bovine α_{1C}-adrenergic receptor provide evidence for its classification as the α_{1A} subtype. Mol. Pharmacol. 46, 823–831.

Piascik, M.T., Smith, M.S., Soltis, E.E., Perez, D.M., 1994. Identification of the mRNA for the novel α_{1D}-adrenoceptor and two other α_1-adrenoceptors in vascular smooth muscle. Mol. Pharmacol. 46, 30–40.

Rimele, T.J., Henry, D.E., Lee, D.K.H., Geiger, G., Heaslip, R.J., Grimes, D., 1987. Tissue-dependent alpha adrenoceptor activity of buspirone and related compounds. J. Pharmacol. Exp. Ther. 241, 771–778.

Satoh, M., Enomoto, K., Niwano, H., Fujimura, H., Toyama, Y., Takayanagi, I., Koike, K., 1998. Regional differences in α_1-adrenoceptor subtypes and mechanisms in rabbit arteries. Eur. J. Pharmacol. 350, 67–73.

Saussy, D.L., Goetz, A.S., King, H.K., True, T.A., 1994. BMY 7378 is a selective antagonist of α_{1D} adrenoceptors (AR): evidence that the vascular α_1 AR are of the α_{1D} subtype. Can. J. Physiol. Pharmacol. 72, 323P, Suppl.

Saussy, D.L., Goetz, A.S., Queen, K.L., King, H.K., Lutz, M.W., Rimele, T.J., 1996. Structure activity relationship of a series of buspirone analogs at alpha-1 adrenoceptors: Further evidence that rat aorta alpha-1 adrenoceptors are of the alpha-1D-subtype. J. Pharmacol. Exp. Ther. 278, 136–144.

Sinanovic, O., Chiba, S., 1987. Responsiveness of skeletal muscle branches of the dog femoral artery to alpha-adrenoceptor agonists before and after cold storage. Arch. Int. Pharmacodyn. Ther. 287, 146–157.

Suzuki, E., Tsujimoto, G., Tamura, K., Hashimoto, K., 1990. Two pharmacologically distinct α_1-adrenoceptor subtypes in the contraction of rabbit aorta: each subtype couples with a different Ca^{2+} signaling mechanism and plays a different physiological role. Mol. Pharmacol. 38, 725–736.

Suzuki, F., Miyamoto, S., Takita, M., Oshita, M., Watanabe, Y., Kakizuka, A., Narumiya, S., Taniguchi, T., Muramatsu, I., 1997. Cloning, functional expression and tissue distribution of rabbit α_{1d}-adrenoceptor. Biochem. Biophys. Acta 1323, 6–11.

Terrón, J.A., Juan, E.R., Hong, E., Villalón, C.M., 1996. Role of α_1-adrenoceptors in the reduction of external carotid blood flow induced by buspirone and ipsapirone in the dog. Life Sci. 58, 63–73.

Testa, R., Distefani, C., Guarneri, L., Poggesi, E., Simonazzi, I., Taddei, C., Leonardi, A., 1995. The α_{1d}-adrenoceptor subtype is involved in the noradrenaline-induced contractioon of rat aorta. Life Sci. 57, 159–163.

Testa, R., Guarneri, L., Angelico, P., Poggesi, E., Taddei, C., Sironi, G., Colombo, D., Sulpizio, A.C., Naselsky, D.P., Hieble, J.P., Leonardi, A., 1997. Pharmacological characterization of the uroselective alpha-1 antagonist Rec 15/2739 (SB 216469): role of the alpha-1L adrenoceptor in tissue selectivity. Part II. J. Pharmacol. Exp. Ther. 281, 1284–1293.

Vargas, H.M., Gorman, A.J., 1995. Vascular alpha-1 adrenergic receptor subtypes in the regulation of arterial pressure. Life Sci. 57, 2291–2308.

Villalobos-Molina, R., López-Guerrero, J.J., Ibarra, M., 1997. α_{1D}- and α_{1A}-adrenoceptors mediate contraction in rat renal artery. Eur. J. Pharmacol 322, 225–227.