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Evidence that α_{1B}-adrenoceptors are involved in noradrenaline-induced contractions of rat tail artery

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Abstract

The present study characterizes the α_1-adrenoceptor subtypes mediating contractions to noradrenaline in isolated ring preparations of rat tail artery. Concentration–response (*E*/[*A*]) curves to noradrenaline were apparently monophasic (pEC_{50} 6.47) but became biphasic in the presence of the selective α_{1A}-adrenoceptor antagonist (±)-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 (B8805-033). Whereas the first phase of contraction to noradrenaline remained nearly unaffected in the presence of B8805-033 (0.03–3 μM), the second phase was concentration-dependently shifted to the right (pK_B 8.06). In the presence of B8805-033 (3 μM), noradrenaline-induced contractions (pEC_{50} 6.55) were antagonized in a competitive manner by prazosin (pK_B 9.24), tamsulosin (pK_B 8.55), 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane (WB 4101; pK_B 7.81), spiperone (pK_B 7.69), 4-amino-2-[4-[1-(benzyloxycarbonyl)-2(*S*)-[[(1,1-dimethylethyl)amino]carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline (L-765,314; pK_B 7.31), 5-methylurapidil (pK_B 6.55), 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY 7378; pK_B 6.43), and 8-[2-(1,4-benzodioxan-2-ylmethylamino)ethyl]-8-azaspiro[4.5]decane-7,9-dione (MDL 73005EF; pK_B 5.71), and were also antagonized by 100 μM chloroethylclonidine. *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine (RS-17053) behaved as a noncompetitive antagonist (apparent pA_2 6.55). Antagonist affinities obtained under these experimental conditions correlated highly with affinities at native and cloned α_{1B}-adrenoceptors. Pretreatment of arterial rings with B8805-033 (3 μM) followed by receptor inactivation with chloroethylclonidine (100 μM) yielded monophasic *E*/[*A*] curves to noradrenaline (pEC_{50} 6.14). Noradrenaline-induced contractions were competitively antagonized by tamsulosin (pK_B 10.32), 5-methylurapidil (pK_B 8.66), RS-17053 (pK_B 8.44), B8805-033 (pK_B 7.87), BMY 7378 (pK_B 6.54), and L-765,314 (pK_B 6.41). Antagonist affinities obtained under these experimental conditions correlated highly with affinities at native and cloned α_{1A}-adrenoceptors. It is concluded that the contraction to noradrenaline in rat tail artery is mediated by both α_{1B}- and α_{1A}-adrenoceptors, each component of contraction being separable by use of selective α_{1A}-adrenoceptor blockade and α_{1B}-adrenoceptor alkylation, respectively.

Keywords: α_1-Adrenoceptor subtype; α_1-Adrenoceptor antagonist; B8805-033; Chloroethylclonidine; Noradrenaline; Tail artery, rat

1. Introduction

Radioligand binding, molecular biology, and isolated tissue experiments have demonstrated that α_1-adrenoceptors are not a homogeneous class of receptors. Currently, classification of α_1-adrenoceptors accommodates three different α_1-adrenoceptor subtypes (α_{1A}, α_{1B}, and α_{1D}), which have also been cloned (for reviews, see Hieble et al., 1995; Guimães and Moura, 2001). A fourth α_1-adrenoceptor, the putative α_{1L}-adrenoceptor, has not been cloned yet and seems to represent a functional phenotype of the α_{1A}-adrenoceptor (Muramatsu et al., 1990; Ford et al., 1996, 1997; Kava et al., 1998). Quantitative expression patterns of the mRNA encoding the three established α_1-adrenoceptor subtypes (α_{1A}, α_{1B}, and α_{1D}) have been determined in numerous vessels (for reviews, see Guarino et al., 1996; Guimães and Moura, 2001). Remarkably, blood vessels can possess mRNA for all three α_1-adrenoceptors. For example, mRNA for α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptor subtypes has been detected in the rat mesentery artery, aorta, pulmonary artery, and tail artery (Xu et al., 1997; Guarino et al., 1996). In addition, the expression of all three adrenoceptor subtypes

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has been demonstrated in a variety of peripheral arteries of the rat using immunohistochemical methods (Hrometz et al., 1999). In contrast to these observations from molecular biological studies, organ bath studies in some vascular preparations have suggested that only one α_1-adrenoceptor subtype seems to be responsible for noradrenaline-induced contraction (Piascik and Perez, 2001). For example, it has been shown that noradrenaline-induced contractions of rat aorta and rat pulmonary artery are predominantly mediated by activation of α_{1D}-adrenoceptors (Kenny et al., 1995; Hussain and Marshall, 1997), whereas α_{1B}-adrenoceptors are involved in the contractile response to noradrenaline in rat mesenteric resistance arteries (Piascik et al., 1997). On the other hand, α_{1A}-adrenoceptor-mediated contractions have been identified in rat renal arteries (Piascik et al., 1997). Rat small mesenteric arteries contracted with noradrenaline, however, show a pharmacology similar to that of the α_{1L}-adrenoceptor (Stam et al., 1999).

The adrenoceptors responsible for noradrenaline-induced contractions in the isolated rat tail artery have been the object of many investigations. Medgett and Langer (1984) were the first who suggested that the rat tail artery is endowed with more than one α_1-adrenoceptor subtype. Other studies have shown that postjunctional α_2-adrenoceptors may also be involved in the contractile response to noradrenaline of this tissue. Postjunctional α_2-adrenoceptors, however, may not play a prominent role in the vasoconstrictor response to noradrenaline in rat tail artery, since relevant contractile responses to selective α_2-adrenoceptor agonists have only been observed in precontracted vessels (Templeton et al., 1989; MacLean and McGrath, 1990).

Data obtained with the α_{1D}-adrenoceptor antagonist, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione; Goetz et al., 1995), and the preferentially α_{1B}-adrenoceptor alkylating agent, chloroethylclonidine (Han et al., 1987), yielded an important advance in the characterization of α_1-adrenoceptors in rat tail artery. The observation that neither BMY 7378 nor chloroethylclonidine (10 μM) affected contractions to noradrenaline suggested that the α_1-adrenoceptor in rat tail artery is not of the D- and B-subtype, respectively (Piascik et al., 1995; Hrometz et al., 1999). In contrast, at higher concentrations of chloroethylclonidine (100 μM), an inhibition of the response to noradrenaline has been reported (Villalobos-Molina et al., 1998).

Correlation analysis between the affinities of a variety of α_1-adrenoceptor antagonists evaluated against the selective α_{1A}-adrenoceptor agonist, *N*-[5-(4-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulfonamide (A-61603; Knepper et al., 1995), in rat tail artery and affinities from radioligand binding studies at cloned α_{1a}-, α_{1b}-, and α_{1d}-adrenoceptors provided further insights into the nature of the subtypes mediating contraction (Lachnit et al., 1997). High affinities for selective α_{1A}-adrenoceptor antagonists such as 5-methylurapidil, *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1*H*-indole-3-ethanamine (RS-17053), and 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid (*N*-[3-(4,4-diphenylpiperidin-1-yl)propyl]amide methyl ester (SNAP 5089) were obtained in rat tail artery, which suggested an involvement of α_{1A}-adrenoceptors in this tissue. It should be emphasized, however, that the *E*/[*A*] curve to noradrenaline in the presence of the selective α_{1A}-adrenoceptor antagonist, RS-17053, was shifted to the right in a biphasic manner, which led the authors to presume that α_{1B}-adrenoceptors might also be involved in the contractile response to noradrenaline (Lachnit et al., 1997).

In this paper, we describe our attempt to present the evidence for an additional involvement of α_{1B}-adrenoceptors in noradrenaline-induced contraction of rat tail artery. For this purpose, functional experiments in the presence of the selective α_{1A}-adrenoceptor antagonist, (±)-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 (B8805-033; Eltze et al., 2001a), and receptor inactivation studies using chloroethylclonidine and several subtype-selective antagonists were performed. Furthermore, we show that the isolated rat tail artery represents a functional assay that can be used to simultaneously study α_{1A}- or α_{1B}-adrenoceptor-mediated effects depending on the experimental conditions. A preliminary report of some of these data has been published previously (Jähnichen et al., 2003).

2. Materials and methods

2.1. Tissue preparation

All experimental procedures carried out in the present study were within the guidelines of the Animals (Scientific Procedures) Act 1986. Male Wistar rats (280–400 g) were killed by decapitation following asphyxiation with CO_2. The ventral caudal artery was quickly removed and cleared from surrounding adipose tissues. A stainless steel wire (diameter 0.3 mm) was inserted into the lumen of the artery to rub off the endothelium. Arterial rings (3–4 mm long) were cut, horizontally suspended between two L-shaped stainless steel hooks (diameter 0.15 mm), and mounted in a 20-ml organ bath filled with modified Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and glucose 11.5. All experiments were conducted in the continuous presence of ascorbate (0.2 mM), cocaine (30 μM), corticosterone (30 μM), propranolol (1 μM), idazoxan (0.1 μM), and methysergide (1 μM) to block noradrenaline oxidation, neuronal and extraneuronal uptake of noradrenaline, β-adrenoceptors, α_2-adrenoceptors, and 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptors, respectively. It should be emphasized that methysergide (1 μM) did not induce a contractile response in rat tail artery when added to the bathing medium. This is consistent with recently reported observations in this tissue (Yoshio et al., 2001). The solution was continuously aerated with 95% O_2/

5% CO_2 and warmed to a constant temperature of 37 °C. Preparations were connected to an isometric force transducer (W. Fleck, Mainz, Germany) attached to a TSE 4711 transducer coupler and a Siemens C 1016 compensograph for the continuous recording of changes in tension.

2.2. Experimental protocol

Following application of 0.75 *g* resting tension, tissues were allowed to stabilize for 45 min during which time the bathing medium was replaced once after 30 min. The resting tension was reestablished twice (after 15 and 30 min). During an equilibration period of 100 min, tissues were stimulated three times with noradrenaline (1 μM) and once with the maximum effective concentration of noradrenaline (100 μM) followed by washout. Preparations producing insufficient contractile force (< 0.2 *g*) following the exposure to noradrenaline were excluded from the experiment. Two to three cumulative *E*/[*A*] curves to noradrenaline were determined on each arterial ring at intervals of 60 min in the absence and presence of B8805-033 (0.03–3 μM). B8805-033 was incubated for 30 min.

2.3. Experiments in the continuous presence of the α_{1A}-adrenoceptor antagonist, B8805-033

The tissues were mounted and equilibrated as described above. Two to three cumulative *E*/[*A*] curves to noradrenaline, phenylephrine, brimonidine, or buspirone were determined on each arterial ring at intervals of 60 min in the continuous presence of the selective α_{1A}-adrenoceptor antagonist, B8805-033 (3 μM), and in the absence and presence of the test antagonist. Antagonists were incubated for 30 min (except prazosin and RS-17053, which were incubated for 60 min).

In other experiments, a cumulative *E*/[*A*] curve to noradrenaline, phenylephrine, brimonidine, or buspirone was determined on each arterial ring in the presence of B8805-033 (3 μM). Following washout, the tissues were incubated for 30 min with chloroethylclonidine (100 μM). After a washout period of 45 min, a second *E*/[*A*] curve to each agonist was determined in the presence of B8805-033 (3 μM).

2.4. Experiments following inactivation of α_{1B}-adrenoceptors

Following tissue preparation, the tissues were exposed to B8805-033 (3 μM) for 50 min and coincubated with chloroethylclonidine (100 μM) for the final 30 min. At a concentration of 3 μM, B8805-033 is expected to occupy >99% of the α_{1A}-adrenoceptor population (based on a pA_2 of 8.40 as observed in rat vas deferens; Eltze et al., 2001a), whereas it would occupy only ~ 30% of the α_{1B}-adrenoceptor population (based on a pA_2 of 5.21 as observed in guinea pig spleen; Eltze et al., 2001a). Preparations were then washed for 15 min and mounted and equilibrated as described above. Two to three cumulative *E*/[*A*] curves to noradrenaline, A-61603, phenylephrine, brimonidine, or buspirone were determined on each arterial ring at intervals of 60 min in the absence or presence of antagonist. Antagonists were incubated for 30 min (except 5-methylurapidil, prazosin, and RS-17053, which were incubated for 60 min, and tamsulosin, which was incubated for 120 min).

2.5. Data presentation and statistical evaluation

Data are presented as mean ± S.E.M. for *n* experiments, using vessels from at least three animals. Agonist concentration–response (*E*/[*A*]) curves were fitted to the Hill equation using an iterative least squares method (GraphPad Prism 4.0; GraphPad Software, San Diego, CA, USA):

$$E = \frac{E_{\max}}{1 + 10^{(-\mathrm{pEC}_{50} - \log[A])n_{\mathrm{H}}}}$$

to provide estimates of the maximum response E_{max} (tension in *g* or percentage of the response to the maximal contractile concentration of noradrenaline observed in the fourth prestimulation or the maximal contractile response in the first *E*/[*A*] curve to noradrenaline), the half-maximum effective concentration pEC_{50} (the negative logarithm of the molar concentration of the agonist producing 50% of the maximum response), and the midpoint slope n_H. To estimate spontaneous changes both in E_{max} and pEC_{50}, two arterial rings were routinely used as controls. The experiments in test preparations were corrected for spontaneous changes as observed in the control preparation.

Antagonist affinities were expressed as either an apparent pA_2 or pK_B value. Apparent pA_2 values were calculated from the following equation:

$$\mathrm{p}A_2 = -\log[B] + \log(r - 1)$$

where [*B*] is the molar concentration of antagonists and *r* is the ratio of agonist EC_{50} measured in the presence and absence of antagonist (Furchgott, 1972).

When minimum criteria for competitive antagonism were satisfied (i.e., the antagonist produced parallel rightward shifts of the *E*/[*A*] curve without attenuation in the maximum response), antagonist affinities were estimated using the method of Arunlakshana and Schild (1959). If the Schild regression line had a slope not differing significantly from 1.00, the slope was constrained to unity. The intercept on the − log antagonist concentration axis provided the estimate of pK_B (Jenkinson et al., 1995).

Where appropriate, differences between means were determined by Student's *t* test after checking the homogeneity of the variances; *P* values < 0.05 were considered to be significant.

2.6. Drugs

The following drugs were obtained as gifts: ketanserin tartrate (Janssen, Beerse, Belgium), L-765,314 (4-amino-2-

[4-[1-(benzyloxycarbonyl)-2(*S*)-[[(1,1-dimethylethyl)amino]carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline) (Merck and Co., Rahway, USA), methysergide hydrogen maleate (Novartis, Basle, Switzerland), (*R*)-phenylephrine (Winthrop, Norderstedt, Germany), and tamsulosin (Dr. M.C. Michel, University of Amsterdam, Amsterdam, The Netherlands). The following drugs were purchased: cocaine hydrochloride and noradrenaline bitartrate from Merck (Darmstadt, Germany). Brimonidine, chloroethylclonidine dihydrochloride, corticosterone, idazoxan hydrochloride, prazosin hydrochloride, (*R*,*S*)-propranolol, and spiperone hydrochloride were from Sigma-Aldrich (Taufkirchen, Germany). A-61603 (*N*-[5-(4-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulfonamide) hydrobromide, BMY 7378 dihydrochloride, buspirone hydrochloride, MDL 73005EF (8-[2-(1,4-benzodioxan-2-ylmethylamino)ethyl]-8-azaspiro[4.5]decane-7,9-dione) hydrochloride, RS-17053 hydrochloride, and WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane) hydrochloride were from Tocris (Bristol, UK). B8805-033 and 5-methylurapidil were synthesized by ALTANA Pharma (Constance, Germany).

All drugs were dissolved in distilled water or dimethylsulfoxide (5-methylurapidil, B8805-033, corticosterone, L-765,314, RS-17053, and tamsulosin) to a 1- or 10-mmol/l stock solution with the following exceptions: prazosin and spiperone were dissolved in ethanol (50%), and brimonidine in warm ethanol (96%). Stock solutions were diluted in distilled water. Final organ bath concentrations of dimethylsulfoxide or ethanol were less than 0.2%.

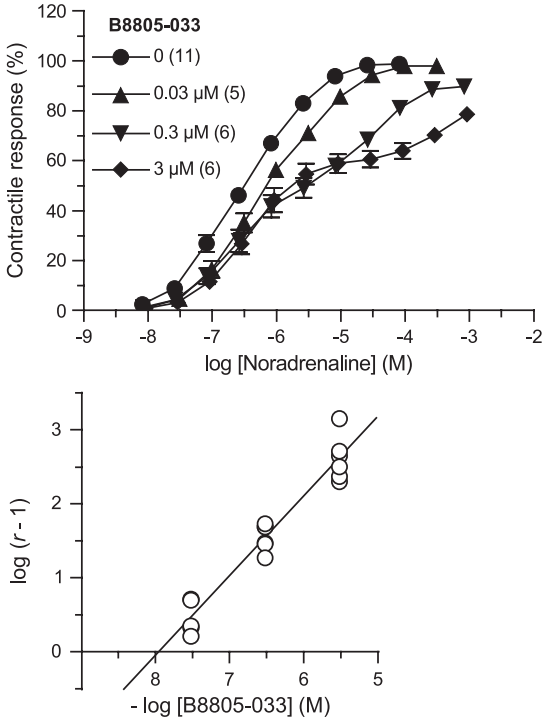

Fig. 1. Antagonism of noradrenaline-induced contraction by B8805-033 in rat tail artery. The upper panel represents cumulative *E*/[*A*] curves to noradrenaline (second or third curves) in the absence and presence of increasing concentrations of B8805-033. Points are mean values (percentage of the maximum response to noradrenaline in the first curve) ± S.E.M. (vertical bars) for the number of tissues indicated in parentheses. The lower panel represents the Schild regression analysis for B8805-033 relating to the second phase of the *E*/[*A*] curve to noradrenaline.

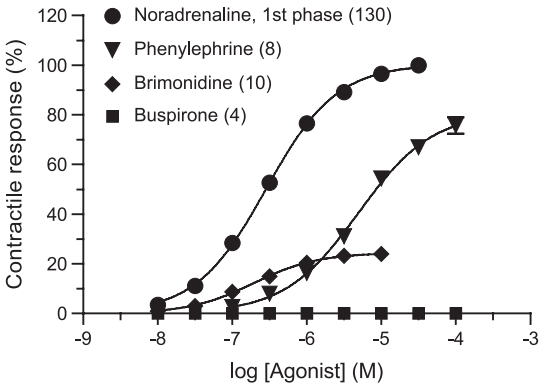

Fig. 2. *E*/[*A*] curves (first curves) to α-adrenoceptor agonists in the continuous presence of B8805-033 (3 μM). The curve to noradrenaline constitutes the first component of contraction in the presence of B8805-033 shown in Fig. 1. Points are mean values (percentage of the response to 100 μM noradrenaline observed in the fourth prestimulation) ± S.E.M. (vertical bars) for the number of tissues indicated in parentheses.

3. Results

3.1. Effect of the selective α_{1A}-adrenoceptor antagonist, B8805-033

Noradrenaline produced concentration-dependent contractions of rat tail arterial rings. The *E*/[*A*] curve to noradrenaline was apparently monophasic ($pEC_{50} = 6.47 \pm 0.03$, $E_{max} = 0.73 \pm 0.04$ g, $n_H = 0.85 \pm 0.02$, $n = 61$) but became biphasic in the presence of the selective α_{1A}-adrenoceptor antagonist, B8805-033 (0.03–3 μM). The first phase of contraction to noradrenaline was nearly resistant to blockade

Table 1
Pharmacological parameters of α-adrenoceptor agonists, inhibitory effects of chloroethylclonidine, and affinity estimates for L-765,314 in the presence of B8805-033 (3 μM)

Agonist	pEC_{50}	E_{max} (%)	Inhibitory effect (%) of choroethylclonidine (100 μM)[a]	pA_2[b] for L-765,314 (0.3 μM)
Noradrenaline	6.55 ± 0.02 (130)	100	83 ± 4 (4)	7.36 ± 0.12 (7)
Phenylephrine	5.31 ± 0.05 (8)	81 ± 3	76 ± 3 (4)	7.44 ± 0.08 (4)
Buspirone	– (4)	0	–	–
Brimonidine	6.64 ± 0.10 (10)	24 ± 3	86 ± 8 (4)	<6.5 (5)

The number of experiments is given in parentheses.
[a] Inhibition of the contractile response at 10 μM agonist.
[b] Apparent pA_2.

by B8805-033. In contrast, the second phase of contraction to noradrenaline was concentration-dependently shifted to the right by B8805-033 (Fig. 1). A three-point Schild regression analysis of the second phase of the $E/[A]$ curve yielded a pK_B of 8.06 ± 0.05 for B8805-033, calculated by the difference of the pEC_{75} (the negative logarithm of the molar concentration of the agonist producing 75% of the maximum response) in the absence and presence of increasing concentrations of B8805-033. The slope of the Schild plot was 1.07 ± 0.07 (not significantly different from unity). The affinity for B8805-033 was in the same concentration range as determined at α_{1A}-adrenoceptors of rat vas deferens (pK_B 8.4), rat cortex

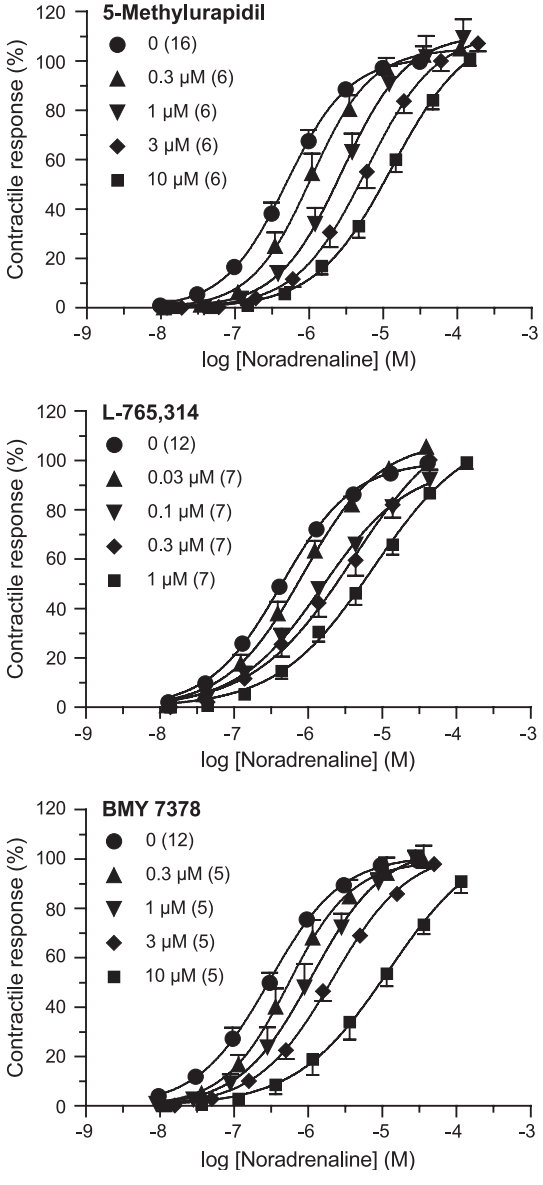

Fig. 3. $E/[A]$ curves (second or third curves) to noradrenaline in the continuous presence of B8805-033 (3 μM) and the presence of increasing concentrations of 5-methylurapidil (top), L-765,314 (middle), and BMY 7378 (bottom), respectively. Points are mean values (percentage of the maximum response to noradrenaline in the first curve) ± S.E.M. (vertical bars) for the number of tissues indicated in parentheses.

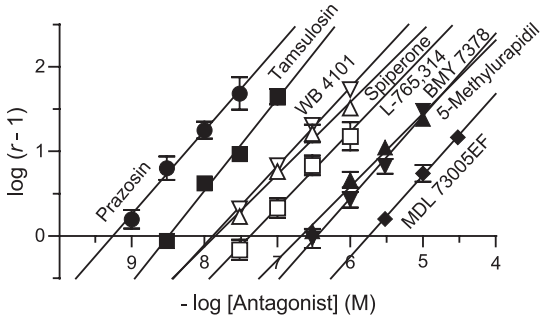

Fig. 4. Schild regression analysis for antagonism of noradrenaline-induced contractions by α_1-adrenoceptor antagonists in the continuous presence of B8805-033 (3 μM). Points are mean ± S.E.M. (vertical bars).

(pK_i 8.7) and cloned human α_{1a}-adrenoceptors (pK_i 7.7) (Eltze et al., 2001a).

3.2. Effect of adrenoceptor agonists and antagonists in the continuous presence of the α_{1A}-adrenoceptor antagonist, B8805-033

The purpose of these experiments was to study the component of the $E/[A]$ curve to noradrenaline that was insensitive to B8805-033 (first component of the curve shown in Fig. 1). In the continuous presence of B8805-033 (3 μM), noradrenaline produced concentration-dependent contractions (pEC_{50} = 6.55 ± 0.02, E_{max} = 0.41 ± 0.02 g, n_H = 0.99 ± 0.01, n = 130). Phenylephrine and brimonidine were also identified as agonists, whereas the putative α_{1D}-adrenoceptor agonist, buspirone (Eltze et al., 1999), failed to exhibit agonist activity (Fig. 2). The contractile responses to noradrenaline and phenylephrine were dramat-

Table 2
Affinity estimates for α_1-adrenoceptor antagonists against noradrenaline in the presence of B8805-033 (3 μM) and correlations to published radioligand binding affinities

Antagonist	Rat tail artery			Radioligand binding affinities[a]		
	pK_B	Slope	n	α_{1a}	α_{1b}	α_{1d}
5-Methylurapidil	6.55 ± 0.04	0.87 ± 0.07	24	8.58	6.97	7.49
BMY 7378	6.43 ± 0.04	0.98 ± 0.08	20	6.37	6.78	8.63
L-765,314	7.31 ± 0.06	0.91 ± 0.11	26	6.38	8.70	7.47
MDL 73005EF	5.71 ± 0.04	0.96 ± 0.11	15	6.10	6.88	8.16
Prazosin	9.24 ± 0.07	0.99 ± 0.12	23	9.42	9.53	9.57
RS-17053[b]	6.55 ± 0.17[c]	–	4	8.95	7.53	7.60
Spiperone	7.69 ± 0.04	0.86 ± 0.07	24	7.03	8.24	7.38
Tamsulosin	8.55 ± 0.04	1.09 ± 0.06	20	10.38	9.50	10.04
WB 4101	7.81 ± 0.02	0.93 ± 0.04	16	9.43	8.22	9.05
r^2				0.41	0.87	0.40
P				>0.05	0.0002	>0.05

[a] Means of radioligand binding affinities (pK_i) at cloned human α_1-adrenoceptor subtypes were taken from Buckner et al. (1996), Eltze et al. (2001a,b), Ford et al. (1997), Goetz et al. (1994), Kenny et al. (1995, 1996), Patane et al. (1998), Saussy et al. (1996), Taniguchi et al. (1997), and Zhang et al. (1999).

[b] Insurmountable antagonist.

[c] Apparent pA_2 calculated at 0.3 μM RS-17053.

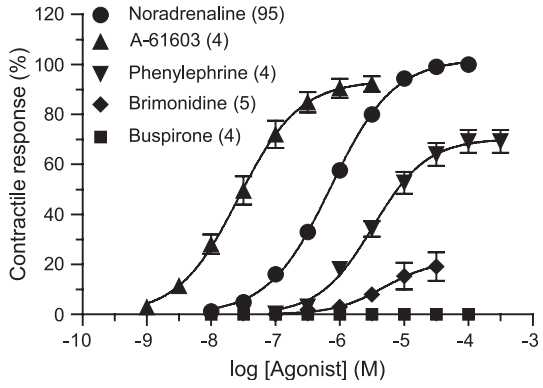

Fig. 5. *E*/[*A*] curves (first curves) to α-adrenoceptor agonists after receptor inactivation with chloroethylclonidine (100 μM) following protection with B8805-033 (3 μM). Points are mean values (percentage of the response to 100 μM noradrenaline observed in the fourth prestimulation) ± S.E.M. (vertical bars) for the number of tissues indicated in parentheses.

ically reduced by chloroethylclonidine (100 μM) and antagonized by L-765,314 (0.3 μM). Brimonidine-induced contractions were sensitive to chloroethylclonidine (100 μM) but not to L-765,314 (0.3 μM) (Table 1).

In the continuous presence of B8805-033 (3 μM), noradrenaline-induced contractions were concentration-dependently antagonized by prazosin, tamsulosin, WB 4101, spiperone, L-765,314, 5-methylurapidil, BMY 7378, and MDL 73005EF without depression of the maximum response. *E*/[*A*] curves to noradrenaline in the absence and presence of 5-methylurapidil, L-765,314, and BMY 7378 are representatively shown in Fig. 3. Schild analysis yielded straight lines with slopes not significantly different from unity (Fig. 4). RS-17053 induced a rightward shift of the *E*/[*A*] curve to noradrenaline and, at higher concentrations, a depression of the maximum response. The apparent pA_2 value of 6.55 (log$r = 0.35 \pm 0.10$) was determined from a single concentration of RS-17053 (0.3 μM) where no depression of the maximum response was observed. Affinity estimates for the antagonists against noradrenaline in the continuous presence of B8805-033 (3 μM) fitted best with radioligand binding data at cloned human α_{1b}-adrenoceptors (Table 2) and with functional affinities (pA_2 values) at guinea pig α_{1B}-adrenoceptors but not with the other subtypes of α_1-adrenoceptors (i.e., α_{1a} and α_{1d} or α_{1A} and α_{1D}, respectively; Fig. 8).

Table 3
Pharmacological parameters of α-adrenoceptor agonists and affinity estimates for B8805-033 after receptor inactivation with chloroethylclonidine (100 μM) following protection with B8805-033 (3 μM)

Agonist	pEC$_{50}$	E_{max} (%)	Apparent pA_2 for B8805-033 (0.1 μM)
Noradrenaline	6.14 ± 0.02 (95)	100	7.84 ± 0.05 (5)
Phenylephrine	5.48 ± 0.03 (4)	70 ± 4	7.95 ± 0.03 (4)
Buspirone	– (4)	0	–
A-61603	7.57 ± 0.09 (4)	93 ± 3	7.77 ± 0.11 (4)
Brimonidine	5.28 ± 0.07 (4)	13 ± 2	7.50 ± 0.2 (5)[a]

[a] pA_2 possibly underestimated since the *E*/[*A*] curve did not reach a plateau at 30 μM.

3.3. Effect of adrenoceptor agonists and antagonists following inactivation of α_{1B}-adrenoceptors

The purpose of these experiments was to study the component of the *E*/[*A*] curve to noradrenaline that was sensitive to B8805-033 (second component of the curve shown in Fig. 1). Selective protection of α_{1A}-adrenoceptors with B8805-033 (3 μM) followed by inactivation with the

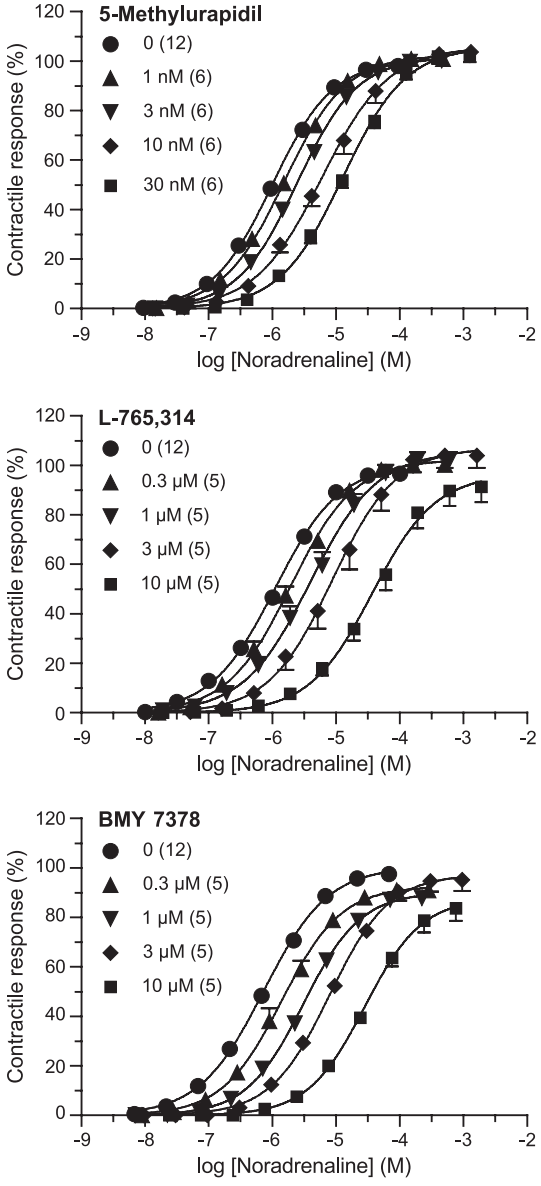

Fig. 6. *E*/[*A*] curves (second or third curves) to noradrenaline after receptor inactivation with chloroethylclonidine (100 μM) following protection with B8805-033 (3 μM) in the presence of increasing concentrations of 5-methylurapidil (top), L-765,314 (middle), and BMY 7378 (bottom), respectively. Points are mean values (percentage of the maximum response to noradrenaline in the first curve) ± S.E.M. (vertical bars) for the number of tissues indicated in parentheses.

preferentially α_{1B}-adrenoceptor alkylating agent, chloroethylclonidine (100 μM), led to a rightward shift of the *E*/[*A*] curve to noradrenaline, a depression of the maximum response, and an increase in the midpoint slope n_H ($pEC_{50} = 6.14 \pm 0.02$, $E_{max} = 0.61 \pm 0.02$ g, $n_H = 0.93 \pm 0.01$, $n = 95$) compared to the curve in the absence of this treatment ($P < 0.05$; see Section 3.1). In addition, A-61603, phenylephrine, and brimonidine elicited contractile responses under these experimental conditions (Fig. 5). *E*/[*A*] curves to noradrenaline, A-61603, phenylephrine, and brimonidine were concentration-dependently shifted to the right by B8805-033 (0.1 μM). The obtained apparent pA_2 values of 7.50–7.95 for B8805-033 (Table 3) were consistent with published antagonist affinities at α_{1A}-adrenoceptors (see Eltze et al., 2001a). It is worth mentioning that the putative α_{1D}-adrenoceptor agonist, buspirone, also lacked agonism under these experimental conditions (see Fig. 5).

Noradrenaline-induced contractions were antagonized by increasing concentrations of tamsulosin, 5-methylurapidil, RS-17053, B8805-033, L-765,314, and BMY 7378. *E*/[*A*] curves to noradrenaline in the absence and presence of 5-methylurapidil, L-765,314 and BMY 7378 are representatively shown in Fig. 6. Schild analysis yielded straight lines with slopes not significantly different from unity for all antagonists except tamsulosin (Fig. 7). Affinity estimates for the antagonists are shown in Table 4. The antagonist properties of tamsulosin (0.03–1 nM) against noradrenaline justify special mention. Schild analysis for tamsulosin yielded a pA_2 of 10.10 ± 0.07 with a slope of the regression line significantly higher than unity (1.34 ± 0.14) indicating insufficient equilibrium between agonist and antagonist at the lowest antagonist concentration (Kenakin, 1993). Schild analysis performed under exclusion of the lowest concentration of tamsulosin (0.03 nM) yielded a pK_B of 10.32 ± 0.05 with a slope of the regression not significantly different from unity (1.17 ± 0.12; Table 4). Affinity estimates for the antagonists against noradrenaline after receptor inactivation with chloroethylclonidine (100 μM) following protection with B8805-033 (3 μM) fitted solely with radioligand binding data at cloned human α_{1a}-adrenoceptors (see Table 4).

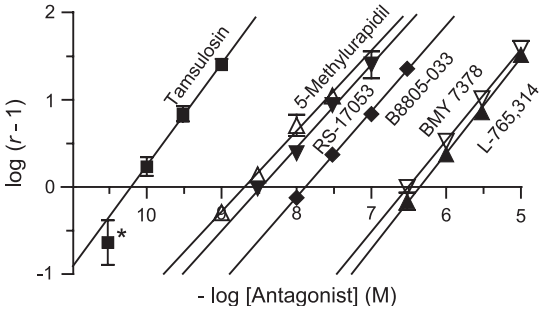

Fig. 7. Schild regression analysis for antagonism of noradrenaline-induced contractions by α_1-adrenoceptor antagonists after receptor inactivation with chloroethylclonidine (100 μM) following protection with B8805-033 (3 μM). Points are mean ± S.E.M. (vertical bars). *Value was excluded from analysis due to insufficient equilibrium between noradrenaline and tamsulosin (0.03 nM; see Results).

Table 4
Affinity estimates for α_1-adrenoceptor antagonists against noradrenaline after receptor inactivation with chloroethylclonidine (100 μM) following protection with B8805-033 (3 μM) and correlations to published radioligand binding affinities

Antagonist	Rat tail artery			Radioligand binding affinities[a]		
	pK_B	Slope	*n*	α_{1a}	α_{1b}	α_{1d}
5-Methylurapidil	8.66 ± 0.04	0.93 ± 0.07	24	8.58	6.97	7.49
B8805-033	7.87 ± 0.02	0.99 ± 0.04	20	7.71	5.16	5.49
BMY 7378	6.54 ± 0.04	1.05 ± 0.06	20	6.37	6.78	8.63
L-765,314	6.41 ± 0.03	1.10 ± 0.06	20	6.38	8.70	7.47
RS-17053	8.44 ± 0.04	0.95 ± 0.07	24	8.95	7.53	7.60
Tamsulosin	10.32 ± 0.05	1.17 ± 0.12	15	10.38	9.50	10.04
r^2				0.98	0.11	0.16
P				0.0002	>0.05	>0.05

[a] Means of radioligand binding affinities (pK_i) at cloned human α_1-adrenoceptor subtypes taken from Buckner et al. (1996), Eltze et al. (2001a,b), Ford et al. (1997), Goetz et al. (1994), Kenny et al. (1995, 1996), Patane et al. (1998), Saussy et al. (1996), Taniguchi et al. (1997), and Zhang et al. (1999).

4. Discussion

4.1. Responses mediated by α_{1A}- and α_{1B}-adrenoceptors

In this study, a highly selective α_{1A}-adrenoceptor antagonist, B8805-033, was employed to characterize the remaining non-α_{1A}-adrenoceptor-mediated contraction to noradrenaline in rat tail artery by use of a number of antagonists showing selectivity between the subtypes α_{1A}, α_{1B}, and α_{1D}, respectively. Additionally, an approach for selective protection of the α_{1A}-adrenoceptor with B8805-033 from alkylation by chloroethylclonidine was used to identify this subtype more thoroughly. Previous studies on rat tail artery have shown that the contractile response to noradrenaline is mediated by more than one α_1-adrenoceptor (Medgett and Langer, 1984; Lachnit et al., 1997). Initially, evidence has been provided that α_{1A}-adrenoceptors are involved in noradrenaline-induced contractions of this tissue (Villalobos-Molina and Ibarra, 1996; Lachnit et al., 1997). *E*/[*A*] curves to noradrenaline of the present study were monophasic in the absence of antagonist and became biphasic in the presence of the selective α_{1A}-adrenoceptor antagonist, B8805-033, indicating contributions from more than one receptor. Recently, a biphasic displacement of the *E*/[*A*] curve to noradrenaline has also been reported in the presence of the selective α_{1A}-adrenoceptor antagonist, RS-17053 (Lachnit et al., 1997).

4.2. Responses mediated by α_{1B}-adrenoceptors

In the presence of B8805-033 (3 μM) noradrenaline, phenylephrine and brimonidine elicited contractile responses in rat tail artery. Only contractions to noradrenaline and

phenylephrine were consistent with the activation of α_{1B}-adrenoceptors since the effects to both agonists were inhibited by the preferentially α_{1B}-adrenoceptor alkylating agent, chloroethylclonidine (100 μM), and the selective α_{1B}-adrenoceptor antagonist, L-765,314 (0.3 μM), respectively. In contrast, brimonidine-induced contractions were only sensitive to chloroethylclonidine (100 μM) but not to L-765,314 (0.3 μM). As chloroethylclonidine has been shown to irreversibly inactivate α_2-adrenoceptors (Michel et al., 1993), contractile responses to brimonidine surmounting the blockade of idazoxan (0.1 μM) can be associated with the activation of postjunctional α_2-adrenoceptors in rat tail artery (Templeton et al., 1989; MacLean and McGrath, 1990).

Buspirone has recently been shown to act as a reliable tool to detect α_{1D}-adrenoceptor-mediated responses in tissues such as rat aorta, mouse aorta, and rat pulmonary artery (Yamamoto and Koike, 2001; Eltze et al., 1999, 2002). In contrast, buspirone displayed no contractile response in tissues endowed with α_{1A}- and α_{1B}-adrenoceptors such as rat vas deferens, guinea pig spleen, and mouse spleen (Eltze et al., 1999, 2002). The observation of the present study that buspirone failed to contract rat tail arteries is also consistent with recently published findings using the α_{1D}-adrenoceptor antagonist, BMY 7378, and argues against the involvement of α_{1D}-adrenoceptors in contractions of this tissue (Piascik et al., 1995). Further evidence that α_{1B}-adrenoceptors contribute to the vasoconstrictor response to noradrenaline in rat tail artery has been provided by estimation of antagonist affinities in the presence of the highly selective α_{1A}-adrenoceptor antagonist, B8805-033, at concentrations (3 μM) to sufficiently block α_{1A}-adrenoceptors (pK_i, pA_2 >7.7) but not yet α_{1B}-adrenoceptors (pK_i, pA_2 <5.6) and α_{1D}-adrenoceptors (pK_i, pA_2 <5.5; Eltze et al., 2001a). Except prazosin, all antagonists investigated are α_1-adrenoceptor subtype-discriminating and were competitive against noradrenaline-evoked contractions (linear Schild regressions with slopes of unity), thereby excluding the involvement of more than one receptor in the contractile response elicited by noradrenaline after selective blockade of α_{1A}-adrenoceptors by B8805-033 (3 μM). The antagonist affinities obtained under these conditions fitted best with radioligand binding data at human cloned α_{1b}-adrenoceptors but excluded α_{1a}- and α_{1d}-adrenoceptors (see Table 2, Fig. 8A–C). Moreover, correlation of the antagonist affinities with those obtained from functional studies at α_{1B}-adrenoceptors in guinea pig spleen ($r^2 = 0.97$, $P < 0.0001$; Fig. 8E) and mouse spleen ($r^2 = 0.96$, $P = 0.0001$) were highly significant. In contrast, correlations with affinities for α_{1A}-adrenoceptors in rat vas deferens ($r^2 = 0.45$, $P > 0.05$) and α_{1D}-adrenoceptors in rat aorta ($r^2 = 0.42$, $P > 0.05$) were not significant (Eltze, 1996; Eltze et al., 2002; Fig. 8D and F). For the sake of completeness, it should be noted that the antagonist affinity for L-765,314 estimated in the present study (pK_B 7.31) was considerably lower than that found in radioligand binding studies (pK_i 8.27 at rat α_{1b}-adrenoceptors and 8.70 at human α_{1b}-adrenoceptors; Patane et al., 1998). On the other hand, the pK_B value of 7.31 for L-765,314 is in good agreement with its affinity at

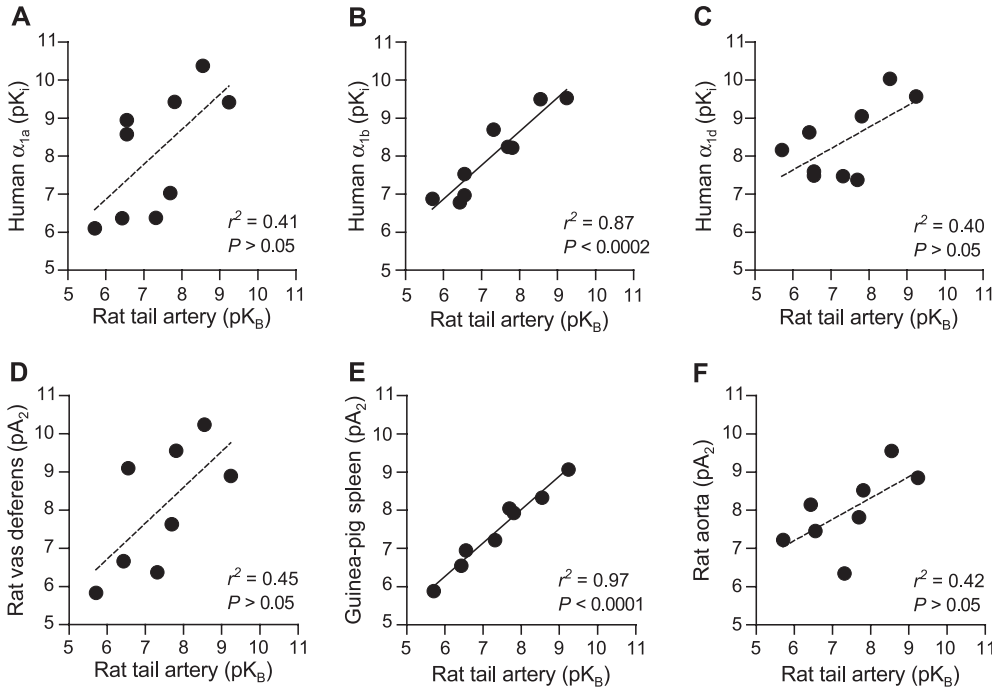

Fig. 8. Correlations of antagonist affinity estimates (pK_B) against noradrenaline in the continuous presence of B8805-033 (3 μM) in rat tail artery and radioligand binding affinities (pK_i) at cloned human α_1-adrenoceptor subtypes as indicated (A–C) and affinities (pA_2) from functional studies at α_{1A}-adrenoceptors in rat vas deferens (D), α_{1B}-adrenoceptors in guinea pig spleen (E), and α_{1D}-adrenoceptors in rat aorta (F).

α_{1B}-adrenoceptors in rat spleen (pA_2 7.55), guinea pig spleen (pA_2 7.22), and rabbit aorta (pA_2 7.27) (Chang et al., 1998; Eltze et al., 2002). Higher affinities in radioligand binding compared to functional studies have also been observed for other antagonists such as 1-[biphenyl-2-yloxy]-4-imino-4-piperidin-1-yl-butan-2-ol (AH11110A) and (+)-cyclazosin at α_{1B}-adrenoceptors (Stam et al., 1998; Eltze et al., 2001b). Such discrepancies may be attributed to diffusion-related temporal inequilibrium in functional studies, which results in underestimation of pK_B (Kenakin, 1993). A further explanation may be that the functional state of the α_{1B}-adrenoceptor could differ from the site(s) mediating the specific binding of the ligands in radioligand binding studies. The involvement of an additional receptor in response to noradrenaline in the presence of B8805-033 is unlikely due to: (i) the correlation analysis at α_{1B}-adrenoceptors in which L-765,314 was the only outlier (Fig. 8B), and (ii) the unity slopes shown for all antagonists including L-765,314 in the present study (Table 2).

4.3. Responses mediated by α_{1A}-adrenoceptors

To examine α_{1A}-adrenoceptor-mediated responses in rat tail artery, we inactivated α_{1B}-adrenoceptors with the preferentially α_{1B}-adrenoceptor alkylating agent, chloroethylclonidine (100 μM). However, it has recently been supposed that data relying only on chloroethylclonidine are not valid (Piascik and Perez, 2001), since this ligand has been reported to inactivate to a certain degree all α_1-adrenoceptors (Michel et al., 1993; Docherty and O'Rourke, 1997; Xiao and Jeffries, 1998). To avoid any interaction of chloroethylclonidine with α_{1A}-adrenoceptors, preparations were preincubated with B8805-033 (3 μM) to selectively protect α_{1A}-adrenoceptors from alkylation when responses mediated by this subtype were intended to be characterized. Under these experimental conditions, noradrenaline, A-61603, phenylephrine, and brimonidine contracted rat tail arteries in a manner consistent with the activation of α_{1A}-adrenoceptors. Contractile responses to the agonists were antagonized by B8805-033 (3 μM), yielding affinity estimates consistent with those typically found to block α_{1A}-adrenoceptor-mediated effects (Eltze et al., 2001a). It should be noted that brimonidine activated α_2- and α_{1A}-adrenoceptors but not α_{1B}-adrenoceptors (see above). Activation of α_2- and α_1-adrenoceptors by brimonidine has recently been demonstrated in human subcutaneous resistance arteries but the contribution of individual α_1-adrenoceptor subtypes was not examined (Jarajapu et al., 2001). The inhibitory effects of a number of subtype-selective α_1-adrenoceptor antagonists against noradrenaline were compatible with α_{1A}-adrenoceptor interaction. Particularly, despite the different selectivities for α_1-adrenoceptor subtypes, the slopes of the Schild plots for all of the antagonists investigated were not significantly different from unity and showed no discontinuity in the regression lines. This is consistent with a single α_1-adrenoceptor-mediating contraction under these conditions. An excellent correlation was found between the antagonist affinities in rat tail artery and radioligand binding affinities at cloned human α_{1a} adrenoceptors, but not with α_{1b}- and α_{1d}-adrenoceptors. In addition, our data correlated well with affinities at α_{1A}-adrenoceptors obtained from other functional studies (rat vas deferens: $r^2 = 0.97$, $P = 0.002$; Eltze, 1996; Eltze et al., 2001a, 2002). It should be mentioned that the affinities of 5-methylurapidil, BMY 7378, RS-17053, and tamsulosin determined under these conditions in rat tail artery (pK_B 8.66, 6.54, 8.44, and 10.32, respectively) were in the same concentration range as determined against the selective α_{1A}-adrenoceptor agonist, A-61603 (pA_2 9.0, 6.3, 9.2, and 11.2, respectively; Lachnit et al., 1997). Moreover, the low affinity of L-765,314 determined under these conditions in rat tail artery (pK_B 6.41) exactly fits to that found for the antagonist against noradrenaline-evoked contractions of this tissue (pK_B 6.38; Chang et al., 1998).

5. Conclusion

A minor but definite role for α_{1B}-adrenoceptors has recently been presented in tail artery using the α_{1B}-adrenoceptor knockout mouse (Daly et al., 2002). Very recently, binding competition experiments using intact tissue segments or membranes from rat tail artery have revealed the coexpression of α_{1A}- and α_{1B}-adrenoceptors in this tissue (Tanaka et al., 2004). The present study is the first functional approach demonstrating that contractile responses to noradrenaline in rat tail artery are mediated by both α_{1A}- and α_{1B}-adrenoceptors. The rat tail artery represents a reliable functional in vitro assay to study α_{1A}- or α_{1B}-adrenoceptor-mediated effects depending on the experimental conditions used.

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