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Functional evidence for an α_{1B}-adrenoceptor mediating contraction of the mouse spleen

Manfrid Eltze *

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

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Abstract

α_1-Adrenoceptor agonists ((−)-adrenaline = (−)-noradrenaline ≫ L-phenylephrine > methoxamine > (−)-(4a*R*,10a*R*)-3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2*H*-naphth[2,3-*b*]-1,4-oxazine (SDZ NVI 085) > cirazoline) evoked contraction of isolated mouse spleen strips, whereas oxymetazoline and indanidine were nearly inactive. Splenic contractions elicited by (−)-noradrenaline were inhibited by chloroethylclonidine (3×10^{-6}–6×10^{-5} M) and partially attenuated by SZL-49 (10^{-7}–10^{-6} M), but remained resistant to (±)-isradipine (10^{-9}–10^{-7} M). The contractions were competitively antagonized by low concentrations of the α_{1B}-adrenoceptor-selective antagonist, spiperone ($pA_2 = 8.29$), but by relatively high concentrations of the α_{1A}-adrenoceptor-selective receptor antagonists, tamsulosin ($pA_2 = 8.62$), 5-methyl-urapidil ($pA_2 = 7.03$), (+)-niguldipine ($pA_2 = 6.26$) and the α_{1D}-adrenoceptor-selective antagonist, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro-[4.5]decane-7,9-dione (BMY 7378) ($pA_2 = 6.76$). Functional antagonist affinities at mouse spleen α_1-adrenoceptors were consistent with those at guinea-pig splenic α_{1B}-adrenoceptors, but not with those of either rat vas deferens α_{1A}- or rat aortic α_{1D}-adrenoceptors. Antagonist affinities at mouse spleen α_1-adrenoceptors correlated also best with published antagonist data on cloned and expressed α_{1b}-adrenoceptors but less well with those for either α_{1a}- or α_{1d}-adrenoceptors. The results provide pharmacological evidence that the α_1-adrenoceptor mediating smooth muscle contraction of mouse spleen is the B subtype.

Keywords: α_1-Adrenoceptor, subtypes A, B and D; Spleen, mouse

1. Introduction

α_1-Adrenoceptors comprise a heterogeneous family (Minneman and Esbenshade, 1994). Initially, only two natively expressed subtypes (α_{1A} and α_{1B}, with uppercase letters) could be distinguished pharmacologically (Morrow and Creese, 1986; Minneman, 1988), whereas three subtypes (α_{1a}, α_{1b} and α_{1d}, with lowercase letters) have been cloned (for a recent review, see Hieble et al., 1995). Meanwhile, convincing evidence has accumulated that the native α_{1D}-adrenoceptor mediates contraction of rat aorta (Kenny et al., 1995; Testa et al., 1995a), a tissue which contains nearly 90% α_{1d}- of the total α_1-adrenoceptor mRNA (Scofield et al., 1995). The native and the cloned subtypes exhibit different affinities for selective antagonists, e.g. tamsulosin, WB 4101 and 5-methyl-urapidil ($\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$), (+)-niguldipine ($\alpha_{1A} \gg \alpha_{1B} = \alpha_{1D}$), benoxathian, SL-89.0591 and naftopidil ($\alpha_{1A} = \alpha_{1D} > \alpha_{1B}$), spiperone ($\alpha_{1B} > \alpha_{1A} = \alpha_{1D}$) or BMY 7378 ($\alpha_{1D} \gg \alpha_{1B} = \alpha_{1A}$) (Han et al., 1987; Gross et al., 1988; Boer et al., 1989; Michel et al., 1989; Forray et al., 1994; Goetz et al., 1994, 1995; Kenny et al., 1995; Testa et al., 1995b). Additionally, the sensitivity of α_1-adrenoceptor-mediated effects to chloroethylclonidine, which inactivates α_{1B}- and α_{1D}-adrenoceptors, or to SZL-49 (prazobind), which completely inactivates α_{1A}- and only partially inactivates α_{1B}- and α_{1D}-adrenoceptors, and to Ca^{2+} channel antagonists, which preferentially attenuate responses to α_{1A}-adrenoceptor stimulation, has served as an additional criterion for differentiating between these subtypes in various tissues (Han et al., 1987; Perez et al., 1991; Pimoule et al., 1995).

For a detailed functional subtype characterization of the α_1-adrenoceptor previously shown to mediate contraction of the mouse spleen by added (−)-noradrenaline (Ignarro and Titus, 1968), we investigated its contractile response to various agonists, including indanidine, which stimulates α_1-adrenoceptors that are solely coupled to Ca^{2+} influx, cirazoline, which needs both entry of extracellular and

* Tel.: 49.7531.842617; fax: 49.7531.842413.

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release of intracellular Ca^{2+} for contraction (Timmermans et al., 1985; Nichols and Ruffolo, 1986), and SDZ NVI 085, which is reported to display higher affinity for α_{1A}- than for α_{1B}-adrenoceptors (Renaud et al., 1991; Büscher et al., 1994). Furthermore, the sensitivity of splenic contractions elicited by (±)-isradipine, and the differentially inactivating actions of chloroethylclonidine and SZL-49 were investigated. Finally, the affinities of a number of α_1-adrenoceptor subtype-discriminating antagonists were determined in mouse spleen and compared with their functional affinities at subtype B in guinea-pig spleen (Eltze, 1994), with subtype A in rat vas deferens (Han et al., 1987; Eltze and Boer, 1992; Kenny et al., 1994; Burt et al., 1995), with subtype D in rat aorta (Saussy et al., 1994; Kenny et al., 1995; Testa et al., 1995a) and with 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 α_{1B}-adrenoceptors to mediate contraction of mouse spleen.

2. Materials and methods

2.1. Mouse spleen

Spleens were obtained from mice (male, 25–30 g, previously anaesthetized by a short exposure to isoflurane, Forene, Abbott). Death by cervical dislocation resulted in less contractile response of the preparations and necessitated prolonged equilibration of the tissue in the baths before the concentration-response curves for added agonists became reproducible (Ignarro and Titus, 1968). The spleen was cut longitudinally into two strips, which were set up in 10-ml organ baths under a resting tension of 0.8 g for recording isometric contractile responses in Krebs-Ringer bicarbonate buffer. The buffer contained (mM) NaCl 120.0, KCl 5.5, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 20.0 and glucose 11.0 and was maintained at 37°C and gassed with 95% O_2-5% CO_2. This solution also 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 in one log unit steps (10^{-8}–10^{-5} M) were generated in the absence or presence of antagonists equilibrated with the splenic strips for 30 min.

2.1.1. Agonist potencies

EC_{50} values of α-adrenoceptor agonists to evoke half-maximal splenic contractions were obtained by non-linear regression analysis of each individual tissue response related to the maximal effect of (−)-noradrenaline (intrinsic activity, i.a. = 1.00) determined in the same splenic strip. In chloroethylclonidine and SZL-49 experiments, the preparations were preincubated with the agents for 45 min, then washed 5 times within 30 min, and the responses to cumulative (−)-noradrenaline were repeated. In (±)-isradipine experiments, contractions in response to (−)-noradrenaline were elicited in the presence of the drug after equilibration with the tissue for 45 min.

2.1.2. Antagonist experiments

Three or four consecutive concentration-response curves of (−)-noradrenaline were recorded at intervals of 45 min until the contractile responses were reproducible. The antagonist was then equilibrated with the tissue strip for 30 min, and the antagonist-induced shift of the agonist curve related to the final control curve was determined for each individual preparation. Generally four different antagonist concentrations were investigated.

2.2. Rat vas deferens, guinea-pig spleen and rat aorta

Isotonic contractions of rat prostatic vas deferens segments or isometric contractions of guinea-pig spleen and rat thoracic aorta in response to cumulatively added (−)-noradrenaline in the absence and presence of antagonists for calculation of pA_2 values at α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, respectively, were elicited as previously described in detail (Eltze and Boer, 1992; Eltze, 1994). Most of the antagonist data were taken from these papers. Additionally, risperidone, indoramin, SL-89.0591, BMY 7378 and naftopidil were evaluated, each compound being equilibrated with the rat vas deferens for 20 min, and with the guinea-pig spleen and rat aorta for 30 min.

2.3. Antagonist affinities and linear regressions

Schild plots were constructed to estimate the pA_2 value and the slope β of the regression line from each experimental series, which generally comprised four different antagonist concentrations. The pA_2 values quoted in Table 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 different tissues.

2.4. Drugs

(−)-(4a*R*,10a*R*)-3,4,4a,5,10,10a-Hexahydro-6-methoxy-4-methyl-9-methylthio-2*H*-naphth[2,3-*b*]-1,4-oxazine hydrogenmalonate (SDZ NVI 085), (±)-isradipine HCl (Sandoz, Basel, Switzerland). L-Phenylephrine HCl (Serva, Heidelberg, FRG). Methoxamine HCl (Wellcome, London, UK). Indanidine HCl (Sgd 101/75) (Siegfried AG, Zofin-

gen, Switzerland). Urapidil HCl, 5-methyl-urapidil, (+)-(*S*)-niguldipine HCl (Byk Gulden). 2-[(3-(4-(5-Chloro-2-methoxyphenyl)piperazin-1-yl)propyl)amino]pyrimidine-4-carboxamine (SL-89.0591), cirazoline (Synthélabo, Bagneux, France). Naftopidil (Boehringer Mannheim, Germany). Flesinoxan HCl (Duphar, Weesp, Netherlands). Ipsapirone HCl (Troponwerke, Cologne, FRG). *rac*-2-(2,6-Dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane HCl (WB 4101), benoxathian HCl, spiperone HCl, chloroethylclonidine diHCl, phentolamine mesylate, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-bicyclo[2,2,2]-octa-2, 5-dienylcarbonyl)-piperazine (SZL-49, prazobind), 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro-[4.5]decane-7,9-dione diHCl (BMY 7378) (RBI, Cologne, Germany). 7-[3-[4-(Diphenylmethoxy)-1-piperidinyl]propyl]-3,7-dihydro-1,3-dimethyl-1*H*-pyrine-2,6-dione (Wy-49.051) (Wyeth-Ayerst, Princeton, USA). Tamsulosin HCl (Yamanouchi, Japan). α-Ethyl-3,4,5-trimethoxy-α-(3-([2-(methoxyphenoxy)ethyl]amino)propyl)benzeneacetonitrile fumarate (HV723) was kindly provided by Prof. I. Muramatsu (Matsuoka, Fukui, Japan). Prazosin HCl (Pfizer, UK). Risperidone (Janssen, Beerse, Belgium). Indoramin HCl (Brenner-Efeka, Münster, Germany). All other drugs were purchased from Sigma (Munich, Germany).

3. Results

3.1. Mouse spleen

3.1.1. Effects of α-adrenoceptor agonists

Cumulative administration of α-adrenoceptor agonists evoked sustained contractions of isolated mouse splenic strips. In most cases, reproducible responses were obtained during the third concentration-response curve and the developed maximum tension evoked by the highest concentration of (−)-noradrenaline (10^{-5} M) amounted to 124 ± 22 mg (mean ± S.D., $n = 84$). Concentration-response curves obtained for the agonists are depicted in Fig. 1. The maximum contractile effect produced by the most potent agonists, (−)-adrenaline and (−)-noradrenaline (intrinsic activity = 1.00), was not reached by L-phenylephrine (intrinsic activity = 0.56). Also methoxamine, SDZ NVI 085 and cirazoline behaved as partial agonists with intrinsic activities of 0.31, 0.46 and 0.22, respectively, whereas contractions elicited by oxymetazoline and indanidine did not reach more than 14% and 5% of maximum, respectively (Table 1).

Table 1
Contractile potencies of α-adrenoceptor agonists in mouse spleen

Agonist	−log EC_{50} (M)	i.a.	*n*
(−)-Adrenaline	6.88 (6.36–7.39)	1.00	12
(−)-Noradrenaline	6.86 (6.25–7.47)	1.00	22
L-Phenylephrine	5.32 (4.60–6.05)	0.56 ± 0.11	11
Methoxamine	4.59 (3.83–5.35)	0.31 ± 0.20	12
SDZ NVI 085	3.69 (3.27–4.11)	0.46 ± 0.16	12
Cirazoline	3.52 (3.22–3.83)	0.22 ± 0.10	11
Oxymetazoline	–	0.14 ± 0.09	12
Indanidine	–	0.05 ± 0.07	12

EC_{50} values for contractions were obtained by non-linear regression analysis of each individual tissue response (means with 95% confidence limits in parentheses). Maximal effects (intrinsic activity, i.a.; means ± S.D.) were related to those elicited by (−)-noradrenaline (i.a. = 1.00).

3.1.2. Studies with chloroethylclonidine, SZL-49 and (±)-isradipine

Treatment of the mouse splenic strips with chloroethylclonidine (3×10^{-6}–6×10^{-5} M) for 45 min progressively depressed the concentration-response curves of (−)-

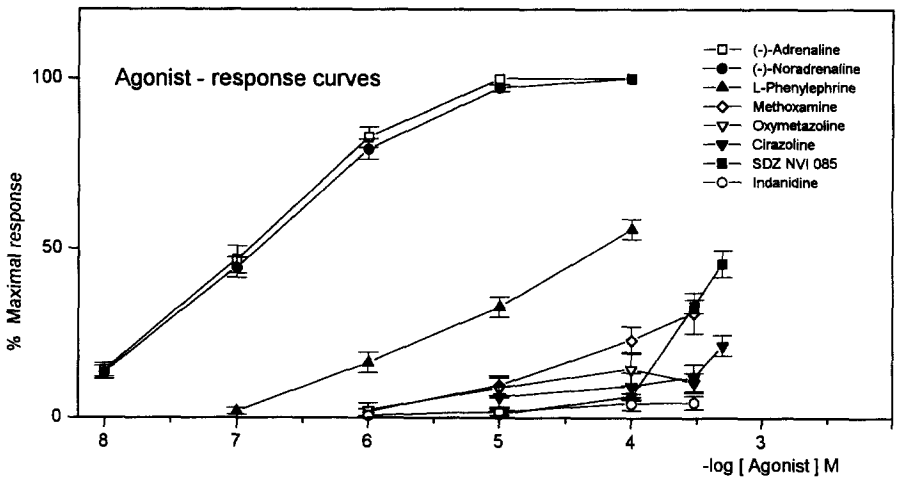

Fig. 1. Concentration-response curves for the contractile effect of α-adrenoceptor agonists, with responses being expressed as a percentage of the maximum contraction elicited by (−)-noradrenaline (= 100%) in the isolated mouse spleen. Given are means ± S.E.M. for $n = 11$–22. Note that oxymetazoline and indanidine had only weak contractile effects not more than 14% and 5% of maximum, respectively.

noradrenaline (Fig. 2, top). At 6×10^{-5} M of chloroethylclonidine, a 65% inhibition of the contraction elicited by 10^{-5} M (−)-noradrenaline was achieved. A 45-min treatment with 10^{-6} M of SZL-49 attenuated the response to 10^{-5} M (−)-noradrenaline by 50% (Fig. 2, middle), whereas the presence of (±)-isradipine (10^{-9}–10^{-7} M) for 45 min did not essentially affect the contractile response to (−)-noradrenaline (Fig. 2, bottom).

3.1.3. Effects of α-adrenoceptor antagonists

The antagonists listed in Table 2 caused parallel shifts to the right of the (−)-noradrenaline concentration-re-

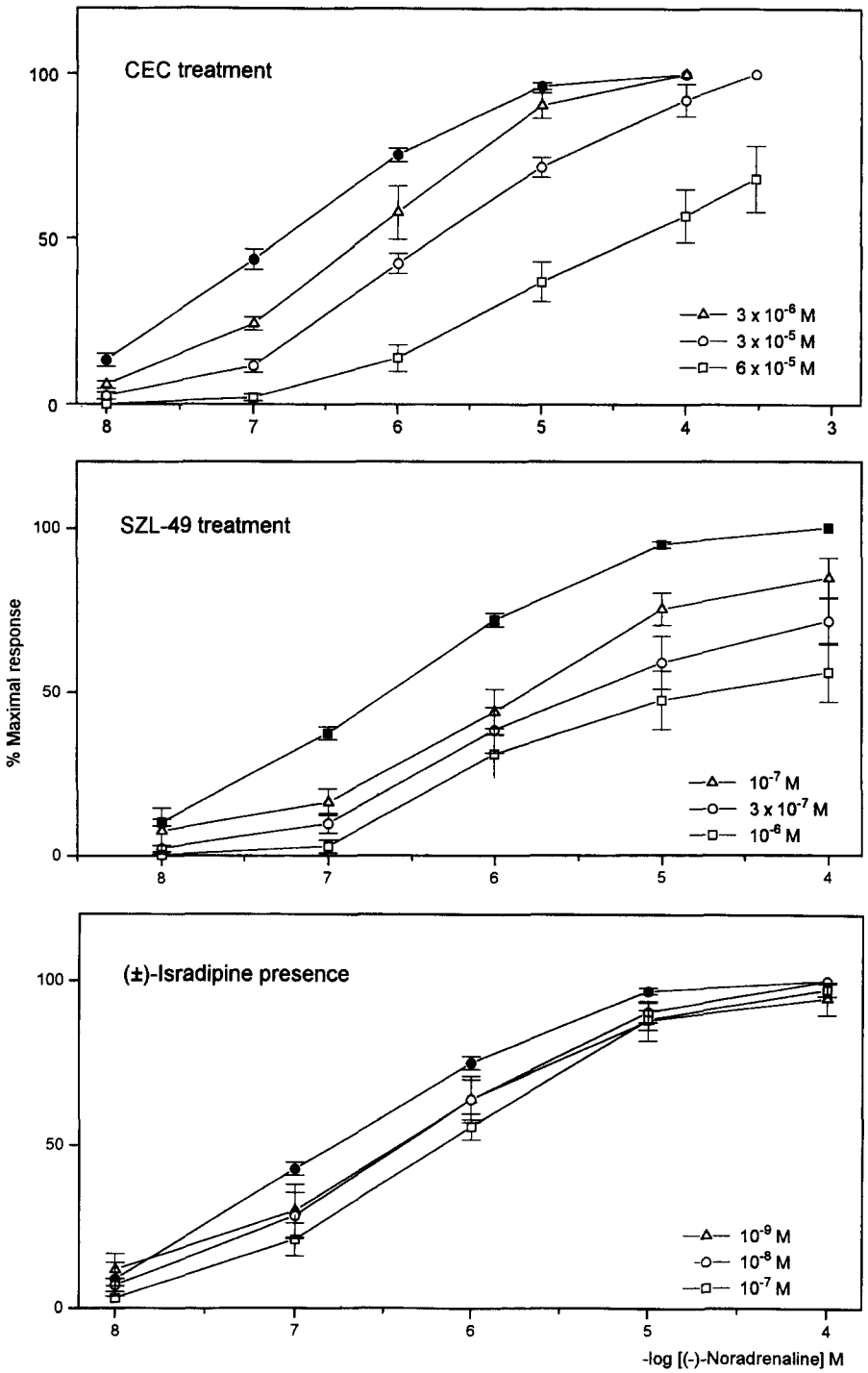

Fig. 2. Effect of chloroethylclonidine (CEC, top) and SZL-49 treatment (middle) for 45 min, as well as the presence of (±)-isradipine for 45 min (bottom), on (−)-noradrenaline-evoked contractions of the isolated mouse spleen (filled circles = control responses, open symbols = after treatment with chloroethylclonidine and SZL-49, or in the presence of (±)-isradipine). Given are means ± S.E.M. of $n = 6$–8.

sponse curve without affecting the maximum contraction caused by the agonist to any noticeable extent, indicating competitive antagonism at α_1-adrenoceptors in mouse spleen. An example of one representative antagonist, spiperone (10^{-8}–3×10^{-7} M), is shown in Fig. 3 (top). The regression lines for a number of antagonists, i.e. WB 4101, spiperone, risperidone, phentolamine, 5-methyl-urapidil and BMY 7378, are shown in Fig. 3 (bottom). Also the Schild plots for all other antagonists listed in Table 2 were linear through the concentration range tested and gave no indication of the presence of multiple subtypes of α-adrenoceptors in this tissue. With the exception of benoxathian and naftopidil, the slopes (β) were not significantly different from unity ($P > 0.05$). The pA_2 values for 18 antagonists, as calculated from constrained regression lines, are listed in Table 2.

3.2. Antagonist affinities in guinea-pig spleen, rat vas deferens and rat aorta

The affinities of a number of other antagonists (risperidone, indoramin, SL-89.0591, BMY 7378, naftopidil and benoxathian), in addition to those already tested previously (Eltze and Boer, 1992; Eltze, 1994) and listed in Table 2, were determined against (−)-noradrenaline-evoked contractions in guinea-pig spleen, rat vas deferens and rat thoracic aorta. These antagonists caused parallel shifts to the right of the (−)-noradrenaline concentration-response

Table 2
pA_2 values (with slopes β of regression lines in parentheses) from constrained Schild plots (β = 1.00) for competitive antagonism at α-adrenoceptors in mouse spleen in comparison with those for α_{1B}-adrenoceptors in guinea-pig spleen, α_{1A}-adrenoceptors in prostatic portions of rat vas deferens and α_{1D}-adrenoceptors in rat thoracic aorta

Antagonist	Spleen, mouse	Spleen, guinea-pig	Vas deferens, rat	Aorta, rat
(1) Prazosin	8.98 ± 0.11 (0.88)	9.07 ± 0.09 (0.99)	8.90 ± 0.13 (0.91)	8.85 ± 0.09 (0.90)
(2) Tamsulosin	8.62 ± 0.17 (1.07)	8.33 ± 0.08 (1.03)	10.24 ± 0.05 (1.19)	9.56 ± 0.07 (0.97)
(3) Spiperone	8.29 ± 0.19 (0.91)	8.05 ± 0.16 (0.77) [a]	7.63 ± 0.03 (0.93)	7.82 ± 0.08 (0.75) [a]
(4) WB 4101	8.28 ± 0.12 (0.84)	7.93 ± 0.05 (1.04)	9.56 ± 0.11 (1.24) [a]	8.53 ± 0.11 (0.87)
(5) HV723	7.59 ± 0.07 (1.19)	7.70 ± 0.09 (1.09)	9.18 ± 0.04 (0.97)	8.14 ± 0.13 (0.79)
(6) 5-Methyl-urapidil	7.03 ± 0.07 (0.93)	6.95 ± 0.17 (0.91)	9.10 ± 0.09 (1.06)	7.03 ± 0.05 (0.89)
(7) Phentolamine	7.37 ± 0.21 (0.90)	6.92 ± 0.20 (1.04)	8.51 ± 0.07 (1.09)	7.23 ± 0.07 (0.76) [a]
(8) Ipsapirone	6.73 ± 0.17 (0.87)	6.45 ± 0.19 (1.16)	7.94 ± 0.05 (1.09)	5.90 ± 0.08 (1.09)
(9) (+)-Niguldipine	6.26 ± 0.16 [b] (–)	6.32 ± 0.11 (–)	– [c]	n.d.
(10) Urapidil	6.52 ± 0.08 (0.86)	6.31 ± 0.12 (0.86)	7.52 ± 0.04 (0.94)	6.16 ± 0.10 (0.80)
(11) Wy-49.051	5.92 ± 0.13 (1.00)	5.89 ± 0.16 (0.93)	7.56 ± 0.06 (1.00)	5.59 ± 0.09 (0.81)
(12) Flesinoxan	5.54 ± 0.08 (0.97)	5.70 ± 0.13 (0.77) [a]	6.99 ± 0.03 (1.00)	5.48 ± 0.06 (0.93)
(13) Benoxathian	7.81 ± 0.18 (1.22) [a]	7.35 ± 0.05 (1.09)	9.35 ± 0.08 (1.04)	8.11 ± 0.09 (0.97)
(14) Risperidone	8.06 ± 0.19 (0.94)	8.12 ± 0.07 (0.97)	9.08 ± 0.11 (1.05)	7.83 ± 0.15 (0.83)
(15) Indoramin	6.69 ± 0.15 (1.11)	6.83 ± 0.12 (0.87)	8.52 ± 0.09 (1.16)	7.03 ± 0.12 (1.14)
(16) SL-89.0591	7.88 ± 0.08 (0.93)	7.54 ± 0.12 (0.93)	8.46 ± 0.08 (0.89)	8.09 ± 0.16 (1.03)
(17) BMY 7378	6.76 ± 0.07 (0.93)	6.55 ± 0.18 (1.02)	6.67 ± 0.15 (0.93)	8.15 ± 0.16 (1.00)
(18) Naftopidil	7.10 ± 0.18 (1.43) [a]	7.05 ± 0.06 (1.00)	8.26 ± 0.15 (0.92)	7.83 ± 0.12 (1.08)

Results are presented as means ± S.E.M. of $n = 6$–8 in spleens from mouse and guinea-pig and $n = 12$–16 in rat vas deferens and rat aorta for each pA_2 determination. Most data for the antagonists (1)–(13) on rat vas deferens, rat aorta and guinea-pig spleen were taken from Eltze and Boer (1992) and Eltze (1994), respectively. [a] Slope β significantly different from unity ($P < 0.05$). [b] Only a concentration of 10^{-6} M was used to calculate pA_2. [c] Not done due to the concomitant strong calcium channel antagonism of (+)-niguldipine (Boer et al., 1989). n.d. = not determined.

curves without affecting the maximal contractile responses to the agonist in these tissues (not shown). Schild plots were linear over the concentration range of the antagonists tested and the slopes of the regression lines were not significantly different from unity ($P > 0.05$).

3.3. Comparison of antagonist data for mouse spleen with affinities at rat vas deferens α_{1A}-, guinea-pig spleen α_{1B}- and rat aortic α_{1D}-adrenoceptors

A highly significant correlation was found ($r = 0.977$, $P < 0.001$; $\beta = 0.93$ not significantly different from 1.00, $P > 0.05$) when we compared the affinities of 18 antagonists, as calculated from their potency to antagonize (−)-noradrenaline-induced mouse splenic contractions, with their affinities for α_{1B}-adrenoceptors of guinea-pig spleen (Fig. 4, middle). However, no significant correlation was obtained by comparing the affinities of 17 antagonists for mouse spleen with the values for rat vas deferens α_{1A}-adrenoceptors ($r = 0.697$, $P > 0.05$; $\beta = 0.70$ significantly different from 1.00, $P < 0.01$; Fig. 4, top). Since only two antagonists, namely tamsulosin and BMY 7378, could differentiate at least 10-fold between mouse splenic α_{1B}- and rat aortic α_{1D}-adrenoceptors, this correlation, inspite of large deviations from the theoretical equality line, looked also apparently good ($r = 0.881$, $P < 00.1$, $n = 17$; $\beta = 1.08$ not significantly different from 1.00, $P > 0.05$; Fig. 4, bottom).

3.4. Comparison of antagonist data for mouse spleen with affinities at cloned and expressed α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors

The affinities of a number of competitive antagonists have now been calculated for membranes from a variety of cells transfected with the cDNAs that express the three

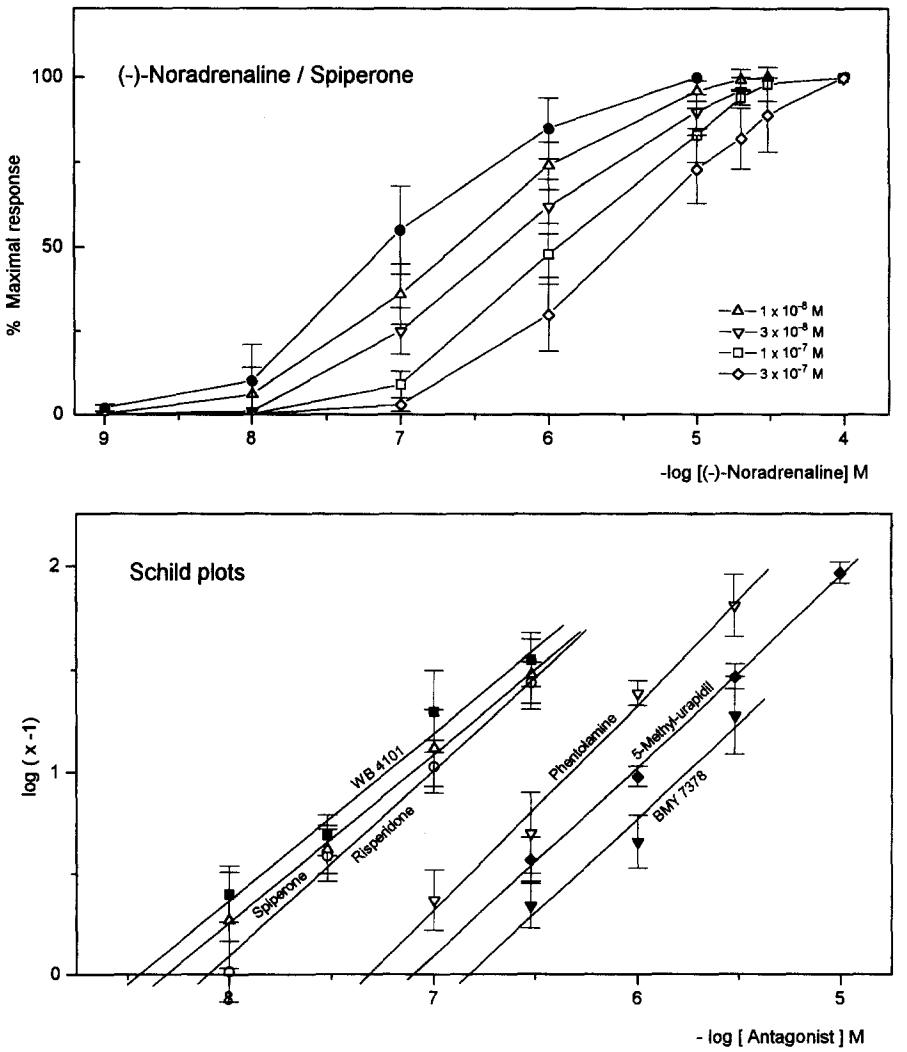

Fig. 3. Top: representative concentration-response curves of (−)-noradrenaline to evoke contraction of the isolated mouse spleen in the absence (filled circles) or presence of increasing concentrations of spiperone (open symbols) equilibrated with the tissue for 30 min. Given are means ± S.D. of $n = 12$ for the control and $n = 4$ in the presence of each concentration of spiperone. Bottom: Schild plots for a selection of antagonists for inhibition of (−)-noradrenaline-evoked contractions of the isolated mouse spleen. Given are means ± S.E.M. of $n = 6–8$.

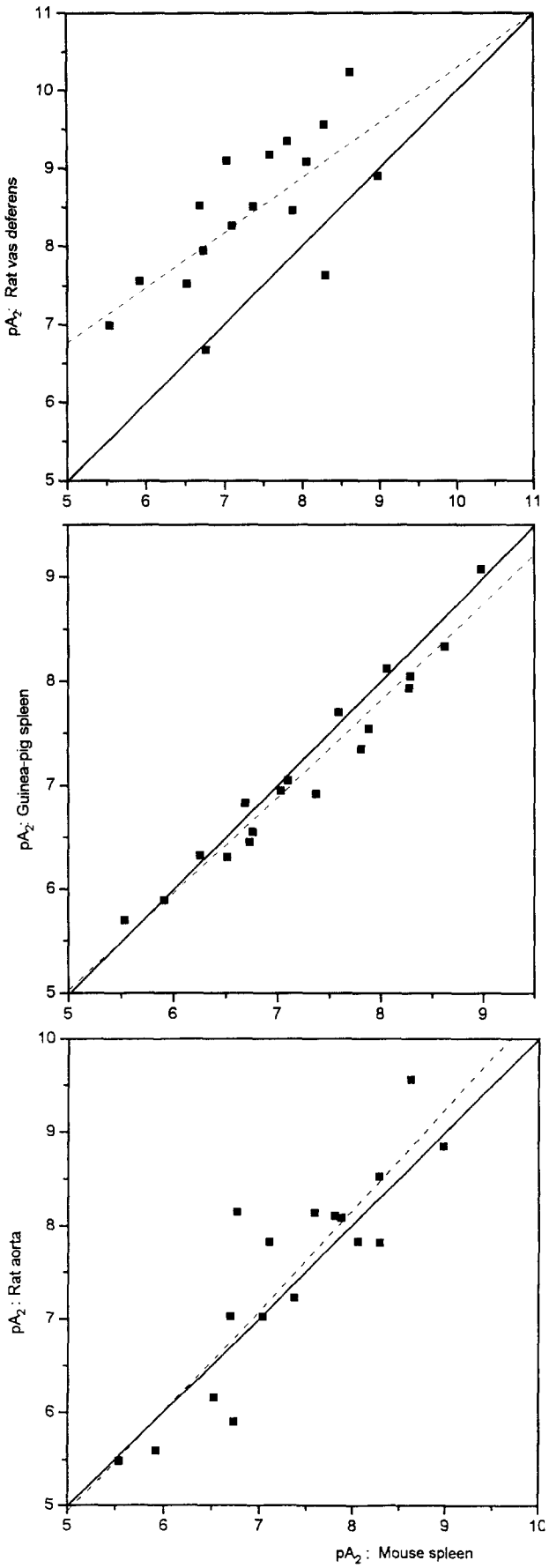

Fig. 4. Relationship between the affinities (pA_2 values) of the antagonists listed in Table 2, determined in mouse spleen, and their functional affinities for α_{1A}-adrenoceptors in rat vas deferens (top), for α_{1B}-adrenoceptors in guinea-pig spleen (middle) and for α_{1D}-adrenoceptors in rat aorta (bottom) listed in Table 2. For receptor identity ($pA_2 = pA_2$), the normal regression line of the experimental data points (dotted) should not deviate significantly from the depicted theoretical equality line (solid).

cloned α_1-adrenoceptor subtypes a, b and d from different sources (for references, see legend of Table 3). In the present study, nine antagonists were selected for which sufficient affinity values at cloned α_1-adrenoceptor subtypes have been published (Table 3). These antagonists display different selectivities, e.g. tamsulosin and 5-methyl-urapidil for subtype a, spiperone for subtype b, and BMY 7378 for subtype d.

An excellent correlation was found ($r = 0.98$, $P < 0.001$; $\beta = 1.17$ not significantly different from 1.00, $P > 0.05$; Fig. 5, middle) when we compared the antagonist affinities from mouse spleen experiments with their average pK_i values for cloned α_{1b}-adrenoceptors. However, weaker correlations were obtained when these affinities were compared with respective values for cloned α_{1a}-adrenoceptors ($r = 0.73$, $P > 0.05$; $\beta = 1.26$ significantly different from 1.00, $P < 0.05$; Fig. 5, top) and for cloned α_{1d}-adrenoceptors ($r = 0.68$, $P < 0.05$; $\beta = 0.77$ significantly different from 1.00, $P < 0.05$; Fig. 5, bottom).

Likewise, the functional affinities (pA_2 values, Table 2) for these nine subtype-discriminating antagonists (listed in Table 3), determined for α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors in rat vas deferens, guinea-pig spleen and rat aorta, respectively, correlated well with average pK_i values calculated from published binding constants for cloned and expressed α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors, respectively (Table 4). Thus the α_1-adrenoceptor subtypes, characterized functionally in these three tissues, exhibit equal affinity for the cloned α_1-adrenoceptor subtypes a, b and d.

4. Discussion

4.1. Agonist studies

Previously, methoxamine and L-phenylephrine have been described as putative α_{1A} subtype-selective agonists (Morrow and Creese, 1986; Tsujimoto et al., 1989). However, the reported selectivities of these agonists, as derived from binding studies, are too low to be unequivocally detectable in functional responses (Lomasney et al., 1991; Minneman et al., 1994). Despite the relative unselectivity of agonists with respect to affinity for α_1-adrenoceptor subtypes, the knowledge that activation of multiple α_1-adrenoceptors by agonists depends on different sources of Ca^{2+} can be useful to identify subtypes. Particularly, the α_1-adrenoceptor agonist indanidine, the response of which

has been suggested to rely on the influx of extracellular Ca^{2+} (Timmermans et al., 1985; Chiu et al., 1987), did not elicit contractile responses of more than 5% in mouse spleen, suggesting that α_1-adrenoceptors coupled closely to Ca^{2+} influx and generally sensitive to stimulation by indanidine, i.e. those of the A subtype, are either totally absent or functionally inactive with regard to contraction of mouse spleen. In accordance with this assumption is the observation that cirazoline, which acts by both releasing Ca^{2+} from intracellular stores and stimulating Ca^{2+} influx (Timmermans et al., 1985; Nichols and Ruffolo, 1986), behaved as a weak partial agonist to evoke contraction in mouse spleen.

Both L-phenylephrine and methoxamine behaved as partial agonists, whereas oxymetazoline evoked an even weaker contractile response in mouse spleen. Interestingly, the different intrinsic activities (in parentheses) obtained in mouse spleen for the catecholamines (−)-noradrenaline (1.00) and (−)-adrenaline (1.00), the phenylethylamines L-phenylephrine (0.56) and methoxamine (0.31), and the imidazolines cirazoline (0.22), oxymetazoline and indanidine (extremely weak responses), are in close agreement with the intrinsic activities of the agonists in stimulating [^{3}H]inositolphosphate formation in HEK 293 cells expressing α_{1b}-adrenoceptors, i.e. both (−)-noradrenaline and (−)-adrenaline (1.00), L-phenylephrine (0.73), methoxamine (0.52), cirazoline (0.16), and both oxymetazoline and indanidine (no measurable response). This contrasts with the intrinsic activity profile of these agonists for cloned and expressed α_{1a}-adrenoceptors, at which both oxymetazoline and indanidine were partial agonists, and also at α_{1d}-adrenoceptors, at which both L-phenylephrine and methoxamine behaved as full agonists (Minneman et al., 1994). Although the relative efficacy of agonists is complicated by the possible presence of receptor reserve and of non-linear relationships between receptor occupancy and response (Ruffolo, 1982), the similarity of agonist intrinsic activities between mouse splenic native α_1-adrenoceptors and functionally expressed α_{1b}-adrenoceptors may serve as a further hint for receptor subtype identity.

The α_1-adrenoceptor agonist, SDZ NVI 085, has been shown to effectively evoke smooth muscle contraction in tissues with an α_{1A}-adrenoceptor supply, e.g. in rabbit ear artery (Nozulak et al., 1992), rat vas deferens and perfused kidney (Eltze and Boer, 1992), with a potency approximately 3-fold weaker than that of (−)-noradrenaline, but has no or only a weak contractile effect in rat thoracic aorta (Eltze and Boer, 1992; Van der Graaf et al., 1995), a tissue very recently characterized as containing functional α_{1D}-adrenoceptors mediating vasoconstriction (Saussy et al., 1994; Kenny et al., 1995; Testa et al., 1995a). In the present study, SDZ NVI 085 was approximately 1000-fold weaker than (−)-noradrenaline to evoke mouse splenic contraction, the potency difference being equal to that observed in guinea-pig spleen (Eltze, 1994). It is feasible that the 40- to 1000-fold lower binding affinity of SDZ NVI 085 at α_{1B}- compared to α_{1A}-adrenoceptors (Renaud et al., 1991; Büscher et al., 1994) accounts for its low potency to elicit contraction in mouse and guinea-pig spleen as compared to its higher potency in tissues with α_{1A}-adrenoceptor supply. Obviously, SDZ NVI 085 is capable of functionally discriminating between various α_1-adrenoceptor subtypes and, in case of a low potency, may at least exclude α_{1A}-adrenoceptors from being involved in a particular response; however, its smaller selectivity for the A over the B subtype (40-fold; Büscher et al., 1994) than initially presumed (1000-fold; Renaud et al., 1991) may not be sufficiently high for a clear α_1-adrenoceptor subtype discrimination in every case, e.g. in binding studies. Moreover, its partial agonistic action both at subtype A in rat vas deferens (Eltze and Boer, 1992) and at subtype B present in guinea-pig spleen (Eltze, 1994) and

Table 3
Comparison of pA_2 values for a number of antagonists in mouse spleen with their published pK_i values for cloned and expressed α_1-adrenoceptor subtypes (means ± S.E.M, with the number of data in parentheses)

Antagonist	Spleen, mouse	Cloned adrenoceptors		
		α_{1a}	α_{1b}	α_{1d}
Tamsulosin	8.62 ± 0.2	10.25 ± 0.2 (5)	8.97 ± 0.2 (5)	9.69 ± 0.3 (5)
Spiperone	8.29 ± 0.2	7.87 ± 0.1 (9)	8.52 ± 0.2 (8)	7.96 ± 0.1 (8)
WB 4101	8.28 ± 0.1	9.37 ± 0.1 (11)	8.09 ± 0.1 (12)	9.12 ± 0.1 (12)
SL-89.0591	7.88 ± 0.1	8.84 ± 0.2 (3)	7.91 ± 0.2 (3)	8.59 ± 0.1 (3)
Benoxathian	7.81 ± 0.2	8.98 ± 0.1 (6)	7.75 ± 0.1 (4)	8.75 ± 0.1 (8)
Phentolamine	7.37 ± 0.2	8.29 ± 0.2 (10)	7.38 ± 0.1 (13)	7.66 ± 0.1 (12)
Naftopidil	7.10 ± 0.2	8.00 ± 0.2 (2)	7.26 ± 0.3 (2)	7.81 ± 0.4 (2)
5-Methyl-urapidil	7.03 ± 0.1	8.66 ± 0.1 (13)	6.87 ± 0.1 (13)	7.46 ± 0.2 (14)
BMY 7378	6.76 ± 0.1	6.28 ± 0.1 (5)	6.51 ± 0.2 (5)	8.44 ± 0.2 (5)

Data were taken from published studies using rat, hamster, bovine and human cloned α_1-adrenoceptor subtypes expressed transiently or stably in a variety of cell lines. References: Perez et al., 1991; Pimoule et al., 1995; Faure et al., 1994; Forray et al., 1994; Goetz et al., 1994; Horie et al., 1994; Kenny et al., 1994; Laz et al., 1994; Saussy et al., 1994; Esbenshade et al., 1995; Goetz et al., 1995; Kenny et al., 1995; Lefevre Borg et al., 1995; Schwinn et al., 1995; Shibata et al., 1995; Testa et al., 1995b.

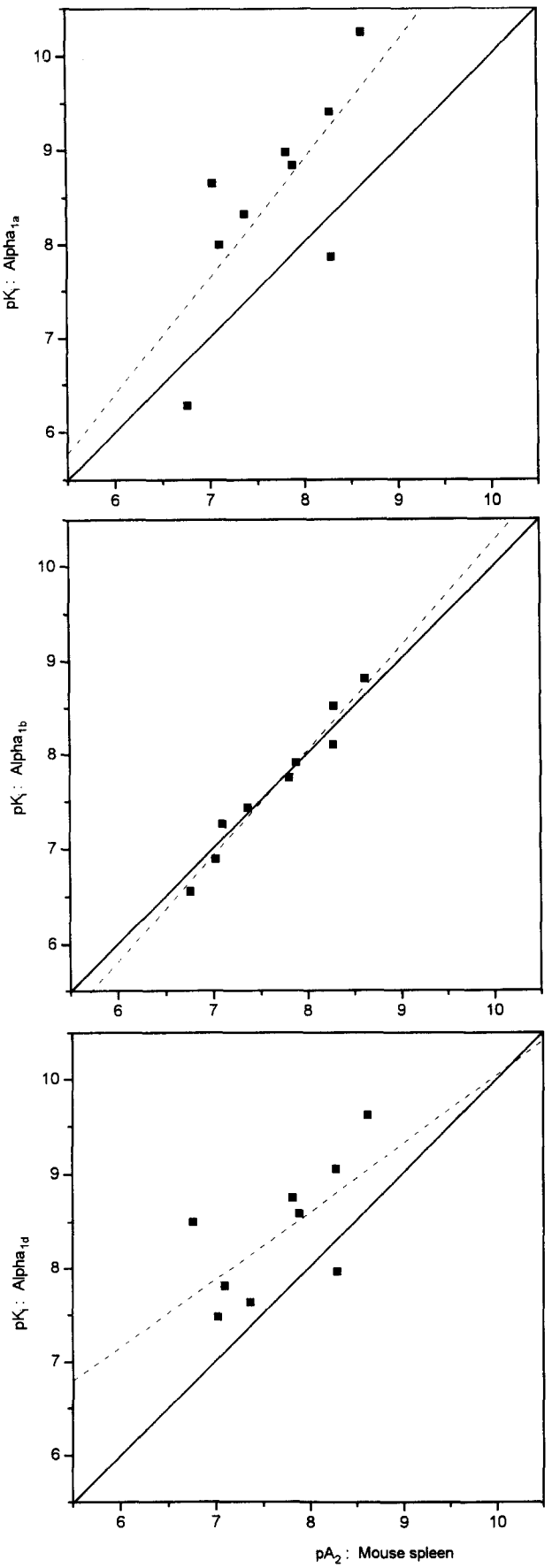

Table 4
Correlation of nine antagonist affinities obtained for native α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors in rat vas deferens, guinea-pig spleen and rat aorta, respectively, with affinities for cloned α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors

Tissue (native receptor)	Cloned adrenoceptors		
	α_{1a}	α_{1b}	α_{1d}
Rat vas deferens (α_{1A})	$r = 0.97$	$r = 0.56$	$r = 0.51$
	$P < 0.001$	$P > 0.05$	$P > 0.05$
	$\beta = 1.00$	$\beta = 0.40$	$\beta = 0.35$
	(> 0.05)	(< 0.01)	(< 0.01)
Guinea-pig spleen (α_{1B})	$r = 0.67$	$r = 0.95$	$r = 0.57$
	$P < 0.05$	$P < 0.001$	$P > 0.05$
	$\beta = 1.22$	$\beta = 1.14$	$\beta = 0.67$
	(< 0.05)	(> 0.05)	(< 0.05)
Rat aorta (α_{1D})	$r = 0.48$	$r = 0.63$	$r = 0.95$
	$P > 0.05$	$P < 0.05$	$P < 0.001$
	$\beta = 0.72$	$\beta = 0.69$	$\beta = 0.95$
	(< 0.05)	(< 0.05)	(> 0.05)

The antagonists listed in Table 3 were used for comparison. The significance of difference from slope $\beta = 1.00$ is indicated in parentheses.

mouse spleen (this study), and at rat renal α_{1A}- and α_{1B}-adrenoceptors (Büscher et al., 1994) may further complicate its use as a discriminating tool.

4.2. Inactivation studies

One of the other criteria used to define the α_1-adrenoceptor subtype in mouse spleen is the sensitivity of the contraction to chloroethylclonidine, which is known to irreversibly inactivate α_{1B}- and to a lesser extent α_{1D}-, but not α_{1A}-adrenoceptors (Minneman, 1988; Han et al., 1987; Pimoule et al., 1995). In the present study, chloroethylclonidine markedly attenuated splenic contractions elicited by (−)-noradrenaline, which points to an α_{1B}-adrenoceptor to be involved in this response. In contrast, SZL-49 has been shown to inactivate α_{1A}-adrenoceptors completely, while causing only partial inactivation of α_{1B}- and α_{1D}-adrenoceptors; however, its usefulness for functional characterization of α_1-adrenoceptor subtypes is controversial (Piascik et al., 1989; Mante and Minneman, 1991; Pimoule et al., 1995). In mouse spleen, SZL-49 at a concentration of 10^{-6} M produced only a maximal 50% depression of the (−)-noradrenaline-evoked contraction, which might at least exclude the α_{1A}-adrenoceptor from being involved in the response.

Fig. 5. Relationship between the affinities (pA_2 values) of a selection of subtype-discriminating antagonists obtained in functional studies with mouse spleen (Table 2) and published binding affinities (pK_i values) for the same antagonists for cloned and expressed α_{1a}-adrenoceptors (top), for α_{1b}-adrenoceptors (middle) and for α_{1d}-adrenoceptors (bottom) listed in Table 3. For receptor identity ($pA_2 = pK_i$), the normal regression line of the experimental data points (dotted) should not deviate significantly from the depicted theoretical equality line (solid).

α_{1B}-Adrenoceptor-mediated contractions are generally considered to be preferentially independent of extracellular Ca^{2+} but rely on the release of stored intracellular Ca^{2+} (Han et al., 1987). At 10^{-7} M of (±)-isradipine, a concentration which is at least 100-fold higher than its affinity for Ca^{2+} channel binding sites (Boer et al., 1989), the contractions of the mouse splenic strip were not affected by the presence of the Ca^{2+} channel antagonist. Similar results were previously obtained from studies on splenic strips from rat (Han et al., 1987) and guinea-pig (Eltze, 1994), the contractions of which are known to be mediated by α_{1B}-adrenoceptors. Thus, also the sensitivity profile of the mouse splenic contractions coincides with that generally found for the B subtype of α_1-adrenoceptors.

4.3. Antagonist studies

The affinities of a number of sufficiently discriminating antagonists for mouse splenic smooth muscle α_1-adrenoceptors were very close to the respective values for previously determined guinea-pig splenic α_{1B}-adrenoceptors (Eltze, 1994). The affinity difference of 18 antagonists determined in the spleens from both species did not exceed a factor of 3, suggesting that splenic contractions elicited by (−)-noradrenaline in both tissues are mediated by the same α_1-adrenoceptor, i.e. the B subtype. In contrast, antagonist affinities in mouse spleen were considerably different from those determined in rat vas deferens and in rat thoracic aorta, two tissues which have convincingly been shown to contain functional α_{1A}- and α_{1D}-adrenoceptors, respectively, which are equivalent to the cloned subtypes a and d, respectively (Eltze and Boer, 1992; Kenny et al., 1994; Saussy et al., 1994; Burt et al., 1995; Kenny et al., 1995; Testa et al., 1995a; this study). Moreover, similar to the study on rat spleen, in which it was attempted to equate the functional α_{1B}- and the cloned α_{1b}-adrenoceptor (Burt et al., 1995), the present study demonstrates that the guinea-pig and mouse spleen α_{1B}-adrenoceptors appear to be the same as the expressed α_{1b}-adrenoceptor clone.

By using these assays for determination of α_1-adrenoceptor subtype affinities, we found prazosin to be unselective, tamsulosin, 5-methyl-urapidil, WB 4101, HV723, phentolamine, benoxathian, indoramin, ipsapirone and flesinoxan more or less selective for subtype A present in rat vas deferens, spiperone moderately selective for subtype B present in mouse and guinea-pig spleen, and BMY 7378 selective for subtype D present in rat aorta. However, 5-methyl-urapidil and phentolamine failed to distinguish between subtypes B and D, whereas BMY 7378 had low but equal affinity for both subtypes A and B. Interestingly, Wy-49.051 had a 30-fold higher affinity for subtype A over both subtypes B and D and thus resembles the selectivity profile of (+)-niguldipine (A ≫ B = D; see Introduction), although with approximately 30-fold lower affinities. Moreover, the claimed high selectivity of risperidone for α_{1B}-adrenoceptors obtained in binding studies (Sleight et al., 1993) could not be confirmed in the present functional experiments. Instead, the compound appeard to be 10-fold selective for the subtype A over both subtypes B and D of α_1-adrenoceptors.

The affinities for a number of antagonists have now been measured on membranes from different cells transfected with the cDNA for each of the three cloned α_1-adrenoceptor subtypes a, b and d from various sources. Nine different subtype-preferring antagonists were selected for comparison with our functional affinity data, including the α_{1a}-adrenoceptor-selective antagonists, tamsulosin and 5-methyl-urapidil, the α_{1b}-adrenoceptor-selective antagonist, spiperone, and the α_{1d}-adrenoceptor-selective antagonist, BMY 7378 (for references, see legend of Table 3). There was a very good correlation between the antagonist affinities for mouse splenic α_1-adrenoceptors and their affinity for cloned α_{1b}-adrenoceptors, suggesting that the functional α_{1B}-adrenoceptor mediating contraction in response to (−)-noradrenaline in mouse spleen exhibits a pharmacological equivalency to the cloned α_{1b}-adrenoceptor. A less close correlation was obtained by comparing the affinities of these antagonists for mouse splenic α_1-adrenoceptors with the respective published values for the compounds for cloned and expressed α_{1a}- and α_{1d}-adrenoceptors. Thus the contraction of the mouse spleen appears unlikely to be mediated via these receptor subtypes.

4.4. Conclusions

The sensitivity of contractions elicited by chloroethylclonidine and their partial inactivation by SZL-49 but their total resistance to Ca^{2+} channel blockade by (±)-isradipine fits into the general hypothesis that (−)-noradrenaline activates α_{1B}-adrenoceptors in mouse spleen to evoke smooth muscle contractions which rely on stored intracellular Ca^{2+}. The affinity characteristics of subtype-discriminating antagonists for α-adrenoceptors in mouse spleen closely resemble those for the native α_{1B}-adrenoceptor in guinea-pig spleen and exhibit a pharmacological equivalency to cloned and expressed α_{1b}-adrenoceptors, but clearly differ from those for native α_{1A}-adrenoceptors in rat vas deferens and native α_{1D}-adrenoceptors in rat aorta, and also from cloned and expressed α_{1a}- and α_{1d}-adrenoceptors. The present study demonstrates that the α_1-adrenoceptor mediating smooth muscle contraction of the mouse spleen, like that found in rat (Han et al., 1987) and guinea-pig (Eltze, 1994), can be best characterized as being of the B subtype and thus constitutes a further unequivocal functional preparation for the study of the α_{1B}-adrenoceptor alone. Consistent with this conclusion is the recent finding of abundant mRNA for the α_{1B}-adrenoceptor in mouse spleen (Cavalli et al., 1995). α_{1B}-Adrenoceptor mRNA is also present in highest concentrations in spleen from rabbit (Schwinn et al., 1991) and man (Price et al., 1994). Thus, all splenic α_1-adrenoceptors of the five species so far investigated belong to the B subtype.

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References

Boer, R., A. Grassegger, C. Schudt and H. Glossmann, 1989, (+)-Niguldipine binds with very high affinity to Ca^{2+} channels and to a subtype of α_1-adrenergic receptors, Eur. J. Pharmacol. 172, 131.

Burt, R.P., C.R. Chapple and I. Marshall, 1995, Evidence for a functional α_{1A}- (α_{1C}-) adrenoceptor mediating contraction of the rat epididymal vas deferens and an α_{1B}-adrenoceptor mediating contraction of the rat spleen, Br. J. Pharmacol. 115, 467.

Büscher, R., P.A. Insel and M.C. Michel, 1994, Is SDZ NVI-085 an α_1-adrenoceptor subtype-selective agonist?, Life Sci. 54, 999.

Cavalli, A., M. Mostardini and S. Cottecchia, 1995, Cloning and tissue distribution of the mouse α_{1B}-adrenergic receptor, Pharmacol. Res. 31 (Suppl.), 49.

Chiu, A.T., J.M. Bozarth and P.B.M.W.M. Timmermans, 1987, Relationship between phosphatidylinositol turnover and Ca^{++} mobilization induced by alpha-1 adrenoceptor stimulation in the rat aorta, J. Pharmacol. Exp. Ther. 240, 123.

Eltze, M. and R. Boer, 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.

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

Esbenshade, T.A., A. Hirasawa, G. Tsujimoto, T. Tanaka, J. Yano, K.P. Minneman and T.J. Murphy, 1995, Cloning of the human α_{1d}-adrenergic receptor and inducible expression of three human subtypes in SK-N-MC cells, Mol. Pharmacol. 47, 977.

Faure, C., C. Pimoule, S. Arbilla, S.Z. Langer and D. Graham, 1994, Expression of α_1-adrenoceptor subtypes in rat tissues: implications for α_1-adrenoceptor classification, Eur. J. Pharmacol. (Mol. Pharmacol. Sect.) 268, 141.

Forray, C., J.A. Bard, J.M. Wetzel, G. Chiu, E. Shapiro, R. Tang, H. Lepor, P.R. Hartig, R.L. Weinshank, T.A. Branchek and C. Gluchowski, 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.

Goetz, A.S., M.W. Lutz, T.J. Rimele and D.L. Saussy, 1994, Characterization of alpha-1 adrenoceptor subtypes in human and canine prostate membranes, J. Pharmacol. Exp. Ther. 271, 1228.

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

Gross, G., G. Hanft and C. Rugevics, 1988, 5-Methyl-urapidil discriminates between subtypes of the α_1-adrenoceptor, Eur. J. Pharmacol. 151, 333.

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

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

Horie, K., A. Hirasawa and G. Tsujimoto, 1994, The pharmacological profile of cloned and stably expressed α_{1B}-adrenoceptor in CHO cells, Eur. J. Pharmacol. (Mol. Pharmacol. Sect.) 268, 399.

Ignarro, L.J. and E. Titus, 1968, The presence of antagonistically acting alpha and beta adrenergic receptors in the mouse spleen, J. Pharmacol. Exp. Ther. 160, 72.

Kenny, B.A., A.M. Naylor, P.M. Greengrass, M.J. Russell, S.J. Friend, A.M. Read and M.G. Wyllie, 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.

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

Laz, T.M., C. Forray, K. E. Smith, J.A. Bard, P.J.J. Vaysse, T.A. Branchek and R.L. Weinshank, 1994, The rat homologue of the bovine α_{1c}-adrenergic receptor shows the pharmacological properties of the classical α_{1A} subtype, Mol. Pharmacol. 46, 414.

Lefevre Borg, F., P. George, S.E. O'Connor, J. Lechaire, A.M. Galzin, S. Arbilla, C. Pimoule, D. Graham and S.Z. Langer, 1995, SL-89.0591: a novel α_1-adrenoceptor antagonist which shows preferential effects on the lower urinary tract, Br. J. Pharmacol. 114 (Suppl.), 419P.

Lomasney, J.W., S. Cotecchia, W. Lorenz, W.-Y. Leung, D.A. Schwinn, T.L. Yang-Feng, M. Brownstein, R.J. Lefkowitz and M.G. Caron, 1991, Molecular cloning and expression of the cDNA for the α_{1A}-adrenergic receptor, the gene for which is located on human chromosome 5, J. Biol. Chem. 266, 6365.

Mante, S. and K.P. Minneman, 1991, The alkylating prazosin analog SZL-49 inactivates both $_{1A}$- and $_{1B}$-adrenoceptors, Eur. J. Pharmacol. (Mol. Pharmacol. Sect.) 208, 113.

Michel, A.D., D.N. Loury and R.L. Whiting, 1989, Identification of a single α_1-adrenoceptor corresponding to the α_{1A}-subtype in rat submaxillary gland, Br. J. Pharmacol. 98, 883.

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

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

Minneman, K.P., T.L. Theroux, S. Hollinger, C. Han and T.A. Esbenshade, 1994, Selectivity of agonists for cloned α_1-adrenoceptors, Mol. Pharmacol. 46, 929.

Morrow, A.L. and I. Creese, 1986, Characterization of α_1-adrenergic receptor subtypes in rat brain: a reevaluation of [^{3}H]WB 4101 and [^{3}H]prazosin binding, Mol. Pharmacol. 29, 321.

Nichols, A.J. and R.R. Ruffolo, 1986, The relationship between alterations in α_1-adrenoceptor reserve by phenoxybenzamine and benextramine and the sensitivity of cirazoline-induced pressor responses to inhibition by nifedipine, Eur. J. Pharmacol. 126, 297.

Nozulak, J., J.M. Vigouret, A.L. Jaton, A. Hofmann, A.R. Dravid, H.P. Weber, H.O. Kalkman and M.D. Walkinshaw, 1992, Centrally acting α_1-adrenoceptor agonists based on hexahydronaphth[2,3-*b*]-1,4-oxazines and octahydrobenzo[*g*]quinolines, J. Med. Chem. 35, 480.

Perez, D.M., M.T. Piascik and R.M. Graham, 1991, Solution-phase library screening for the identification of rare clones: isolation of an α_{1D}-adrenergic receptor cDNA, Mol. Pharmacol. 40, 876.

Piascik, M.T., B.T. Butler, J.W. Kusiak, J. Pitha and J.R. Holtman, 1989, Effect of an alkylating analog of prazosin on α_1-adrenoceptor subtypes and arterial blood pressure, J. Pharmacol. Exp. Ther. 251, 878.

Pimoule, C., S.Z. Langer and D. Graham, 1995, Further evidence that the classical α_{1A}- and cloned α_{1c}-adrenoceptors are the same subtype, Eur. J. Pharmacol. (Mol. Pharmacol. Sect.) 290, 49.

Price, D.T., R.J. Lefkowitz, M.G. Caron, D. Berkowitz and D.A. Schwinn, 1994, Localization of mRNA of three distinct α_1-adrenergic receptor subtypes in human tissues: implication for human α-adrenergic physiology, Mol. Pharmacol. 45, 171.

Renaud, A., S. Nishino, W.C. Dement, C. Guilleminault and E. Mignot, 1991, Effects of SDZ NVI 085, a putative subtype-selective α_1-agonist, on canine cataplexy, a disorder of rapid eye movement sleep, Eur. J. Pharmacol. 205, 11.

Ruffolo, R.R., 1982, Important concepts of receptor theory, J. Auton. Pharmacol. 2, 277.

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

Schwinn, D.A., S.O. Page, J.P. Middleton, W. Lorenz, S.B. Ligget, K. Yamamoto, E.G. Lapetina, M.G. Caron, R.J. Lefkowitz and S. Cotecchia, 1991, The α_{1C}-adrenergic receptor: characterization of signal transduction pathways and mammalian tissue heterogeneity, Mol. Pharmacol. 40, 619.

Schwinn, D.A., G.I. Johnston, S.O. Page, M.J. Mosley, K.H. Wilson, N.P. Worman, S. Campbell, M.D. Fidock, L.M. Furness, D.J. Parry-Smith, B. Peter and D.S. Bailey, 1995, Cloning and pharmacological characterization of human alpha-1 adrenergic receptors: sequence corrections and direct comparison with other species homologues, J. Pharmacol. Exp. Ther. 272, 134.

Scofield, M.A., F. Liu, P.W. Abel and W.B. Jeffries, 1995, Quantification of steady state expression of mRNA for alpha$_1$-adrenergic receptor subtypes using reverse transcription and a competitive polymerase chain reaction, J. Pharmacol. Exp. Ther. 275, 1035.

Shibata, K., R. Foglar, K. Horie, K. Obika, A. Sakomoto, S. Ogawa and G. Tsujimoto, 1995, KMD-3213, a novel, potent, α_{1a}-adrenoceptor selective antagonist: characterization using recombinant human α_1-adrenoceptors and native tissues, Mol. Pharmacol. 48, 250.

Sleight, A.J., W. Koek and D.C.H. Biggs, 1993, Binding of antipsychotic drugs at α_{1A}- and α_{1B}-adrenoceptors: risperidone is selective for the α_{1B}-adrenoceptor, Eur. J. Pharmacol. 238, 407.

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

Testa, R., C. Taddei, E. Poggesi, C. Distefani, S. Cotecchia, J.P. Hieble, A.C. Sulpizio, D. Naselsky, D. Bergsma, C. Ellis, A. Swift, S. Ganguly, R.R. Ruffolo and A. Leonardi, 1995b, Rec 15/2739 (SB 216469): a novel prostate selective α_1-adrenoceptor antagonist, Pharmacol. Commun. 6, 79.

Timmermans, P.B.M.W.M., M.J.M.C. Thoolen, M.-J. Mathy, B. Wilfert, A. De Jonge and P.A. Van Zwieten, 1985, Effects of the irreversible α-adrenoceptor antagonists phenoxybenzamine and benextramine on the effectiveness of nifedipine in inhibiting α_1- and $_2$-adrenoceptor mediated vasoconstriction in pithed rats, Naunyn-Schmiedeberg's Arch. Pharmacol. 329, 404.

Tsujimoto, G., A. Tsujimoto, E. Suzuki and K. Hashimoto, 1989, Glycogen phosphorylase activation by two different α_1-adrenergic receptor subtypes: methoxamine selectively stimulates a putative α_1-adrenergic receptor subtype (α_{1A}) that couples to Ca^{2+} influx, Mol. Pharmacol. 36, 166.

Van der Graaf, P.H., S. Apaydin and P.R. Saxena, 1995, The α_1-adrenoceptor agonist, SDZ NVI-085, behaves as a potent, competitive antagonist of 5-hydroxytryptamine-induced contraction of rat aorta, Eur. J. Pharmacol. 287, 309.