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# Failure of AH11110A to functionally discriminate between $\alpha_1$-adrenoceptor subtypes A, B and D or between $\alpha_1$- and $\alpha_2$-adrenoceptors

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**Abstract**

The potency of the putatively $\alpha_{1B}$-adrenoceptor selective drug, 1-[biphenyl-2-yloxy]-4-imino-4-piperidin-1-yl-butan-2-ol (AH11110A), to antagonize contraction upon stimulation of $\alpha_{1A}$-adrenoceptors in rat vas deferens and rat perfused kidney, $\alpha_{1B}$-adrenoceptors in guinea-pig spleen, mouse spleen and rabbit aorta, and $\alpha_{1D}$-adrenoceptors in rat aorta and pulmonary artery was evaluated and compared to that of a number of subtype-discriminating antagonists. *N*-[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxamide (Rec 15/2739) and (±)-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) were confirmed as selective for $\alpha_{1A}$-adrenoceptors, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY 7378), 8-[2-(1,4-benzodioxan-2-ylmethylamino)ethyl]-8-azaspiro[4.5]decane-7,9-dione (MDL 73005EF), and cystazosin were found to be selective for $\alpha_{1D}$-adrenoceptors, whereas spiperone was weakly selective for $\alpha_{1B}$-over $\alpha_{1A}$-adrenoceptors. However, from the functional affinity profile obtained for AH11110A at $\alpha_{1A}$-adrenoceptors ($pA_2 = 6.41$ in rat vas deferens), $\alpha_{1B}$-adrenoceptors ($pA_2 = 5.40–6.54$) and $\alpha_{1D}$-adrenoceptors ($pA_2 = 5.47–5.48$), the affinity and presumed selectivity previously obtained for AH11110A in radioligand binding studies at native $\alpha_{1B}$- and cloned $\alpha_{1b}$-adrenoceptors ($pK_i = 7.10–7.73$) could not be confirmed. Additionally, AH11110A enhanced the general contractility of rat vas deferens, produced a bell-shaped dose–response curve of vasodilation in perfused rat kidney, and its antagonism in most other tissues was not simply competitive. The affinity of AH11110A for prejunctional $\alpha_2$-adrenoceptors in rabbit vas deferens ($pA_2 = 5.44$) was not much lower than that displayed for $\alpha_1$-adrenoceptor subtypes, revealing that AH11110A, besides $\alpha_1$-adrenoceptors, also interacts with $\alpha_2$-adrenoceptors, and thus may be unsuitable for α-adrenoceptor subtype characterization, at least in smooth muscle containing functional studies. 

*Keywords:* $\alpha_1$-Adrenoceptor; Subtype A, B and D; $\alpha_2$-Adrenoceptor; AH11110A; Selectivity, functional experiment

## 1. Introduction

$\alpha_1$-Adrenoceptors comprise a heterogeneous family (Minneman and Esbenshade, 1994). At present, three natively expressed subtypes ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$, with uppercase letters) can be distinguished pharmacologically and exhibit equivalency to cloned and expressed $\alpha_{1a}$-, $\alpha_{1b}$- and $\alpha_{1d}$-adrenoceptors (with lowercase letters) in various tissues (Hieble et al., 1995). Increasing effort is currently put into the design of subtype-selective compounds in order to achieve tissue or organ selective $\alpha_1$-adrenoceptor blockade. Although a number of antagonists selective for native and cloned $\alpha_{1A}$- and $\alpha_{1D}$-adrenoceptors is now available, e.g. WB 4101, (+)-niguldipine, 5-methyl-urapidil, tamsulosin, Rec 15/2739, RS-17053, KMD-3213 and B8805-033 for subtype A (Han et al., 1987; Gross et al., 1988; Boer et al., 1989; Forray et al., 1994; Eltze et al., 1996; Ford et al., 1996; Testa et al., 1997; Murata et al., 1999), or BMY 7378, MDL 73005EF and cystazosin for subtype D (Goetz et al., 1995; Saussy et al., 1996; Bolognesi et al., 1998), the lack of antagonists sufficiently selective for the subtype B is a persistent problem in $\alpha_1$-adrenoceptor characterization. Presently, spiperone is the only antagonist known for retaining a moderate selectivity (approximately 5-fold) for native or cloned $\alpha_{1B}$-over $\alpha_{1A}$-adrenoceptors both in radioligand and functional experiments, whereas its

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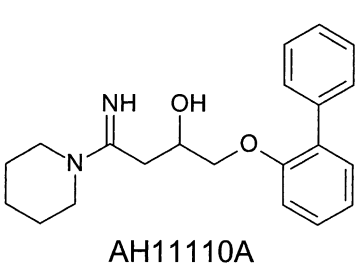
selectivity for subtype B over D at best amounts to a factor of three (Michel et al., 1989; Blue et al., 1995; Kenny et al. 1995; Schwinn et al., 1995; Eltze et al., 1999). The presumed B-subtype selectivity of other antagonists, e.g. risperidone (Sleight et al., 1993) and cyclazosin or its (+)-enantiomer (Giardina et al., 1995, 1996), initially found in binding studies, however, could not be confirmed in subsequent functional experiments (Eltze, 1996a; Stam et al., 1998). In contrast, the quinazoline compound, L-765,314, has been shown to be B-subtype selective both in binding and functional experiments (Chang et al., 1998).

Recently, a novel $\alpha_{1B}$-adrenoceptor-selective compound with no direct chemical resemblance to other $\alpha_1$-adrenoceptor antagonists, 1-[biphenyl-2-yloxy]-4-imino-4-piperidin-1-yl-butan-2-ol HCl (AH11110A; Fig. 1), has been characterized through binding experiments with native or cloned $\alpha_1$-adrenoceptor subtypes in different animal tissues (rat, hamster and bovine) and human tissues, displaying an approximately 10- and 20-fold selectivity to subtype B(b) relative to A(a) and D(d), respectively, and an affinity rank order at these subtypes of B(b) > A(a) > D(d) (King et al., 1994; Giardina et al., 1996; Saussy et al., 1996). Presently, AH11110A is listed as the only recommended selective antagonist for this subtype (see Alexander and Peters, 2000), but, except for its functional affinity at $\alpha_{1D}$-adrenoceptors in rat aorta ($pA_2 = 6.29$; King et al., 1994), further proof of its general utility for the characterization of $\alpha_1$-adrenoceptor subtypes is missing.

Therefore, the present study aims at the assessment of the presumed selectivity of AH11110A at different $\alpha_1$-adrenoceptor subtypes in functional experiments, using at least two assays for each subtype, namely rat vas deferens and perfused kidney for subtype A (Han et al., 1987; Eltze et al., 1991; Eltze and Boer, 1992; Kenny et al., 1994), guinea-pig spleen, mouse spleen and rabbit aorta for subtype B (Eltze 1994, 1996b; Muramatsu et al., 1998) and rat aorta and pulmonary artery for subtype D (Kenny et al., 1995; Hussain and Marshall, 1997; Eltze et al., 1999). Since AH11110A has also been reported to display micromolar affinity ($pK_i = 5.54$) at rat cortical $\alpha_2$-adrenoceptor binding sites (Giardina et al., 1996), we additionally determined its functional antagonist activity at prejunctional $\alpha_2$-adrenoceptors located at adrenergic nerve endings in the field-stimulated rabbit vas deferens (Alabaster et al., 1986).




AH11110A

Fig. 1. Chemical structure of AH11110A.

## 2. Materials and methods

### 2.1. Rat vas deferens and perfused rat kidney: $\alpha_{1A}$-adrenoceptors

Prostatic portions of vas deferens taken from Sprague–Dawley rats (180–250 g) were set up in 20-ml organ baths containing Tyrode solution plus $10^{-5}$ M cocaine, maintained at 37°C and gassed with a mixture of 95% $O_2$–5% $CO_2$. Cumulative concentration–response curves of isotonic contractions to cumulatively added noradrenaline ($10^{-7}$–$2 \times 10^{-4}$ M) were performed in the absence or presence of different antagonist concentrations equilibrated with the tissue for 20 min, when the control response curves had been reproducible and stable after four repetitions (Eltze et al., 1991). In further experiments, the effect of AH11110A, equilibrated with the rat vas deferens for 20 min, on cumulative concentration–response curves of KCl (15–75 mM) was investigated. In these experiments, cocaine was omitted in the nutrient solution.

The potency of $\alpha_1$-adrenoceptor antagonists to attenuate noradrenaline-evoked vasoconstriction was evaluated in isolated kidneys taken from male normotensive rats (Sprague–Dawley, 390–420 g) perfused at a constant pressure of 100 cm $H_2O$ with Tyrode (37°C) and gassed with 95% $O_2$–5% $CO_2$. The prerenal perfusate flow was measured continuously using an electromagnetic flowmeter. After perfusion of the kidney without any drug for 30 min, during which vascular flow stabilized at $15.4 \pm 1.2$ ml/min (mean ± S.D., $n = 31$), the kidney was continuously perfused with $6 \times 10^{-7}$ M noradrenaline, which reduced renal perfusion flow by about 70–80%. Once the vasoconstriction had stabilized, increasing doses of the test drugs (100 μl aqueous bolus) were injected within 2 s into the renal inflow tract and the resulting vasodilation was recorded. The decrease in renal flow obtained by perfusion of the kidney with noradrenaline in the absence of the test substance was taken 100%, and the percent reversal of this effect following injection of increasing doses of the antagonist was calculated for determination of their half-maximal vasodilatory effect ($-\log ED_{50}$; Eltze et al., 1991).

### 2.2. Guinea-pig spleen, mouse spleen and rabbit aorta: $\alpha_{1B}$-adrenoceptors

Spleens were obtained from male guinea-pigs (350–400 g, killed by a blow on the head and exsanguination) or male mice (25–30 g, previously anesthetized by a short exposure to isoflurane, Forene®, Abbott). The spleens were longitudinally cut into six and two strips, respectively, and were set up in 10-ml organ baths under a resting tension of 1.0 and 0.8 g, respectively, for recording isometric contractile responses in Krebs–Ringer bicarbonate buffer maintained at 37°C and gassed with 95% $O_2$–5% $CO_2$, additionally containing $3 \times 10^{-7}$ M desipramine,

$3 \times 10^{-5}$ M corticosterone and $10^{-6}$ M propranolol. The contractions in response to cumulative administration of noradrenaline (half-log unit steps from $10^{-8}$–$3 \times 10^{-4}$ M in guinea-pig spleen; one-log unit steps from $10^{-8}$–$10^{-4}$ M in mouse spleen) were generated in the absence or presence of antagonists equilibrated with the splenic strips for 30 min, when the control response curves had been reproducible and stable after three repetitions (Eltze, 1994, 1996b).

Similarly, ring preparations of the thoracic aorta from male New Zealand white rabbits (2.5–3.0 kg, Charles River, Kisslegg, Germany; killed by exsanguination after the animals had been anesthetized with pentobarbital sodium, 60 mg/kg, i.v.) were mounted in organ baths under a resting tension of 1.5 g in Krebs–Ringer bicarbonate buffer maintained at 37°C and gassed with 95% $O_2$–5% $CO_2$, additionally containing $3 \times 10^{-7}$ M desipramine, $3 \times 10^{-5}$ M corticosterone, $10^{-6}$ M propranolol and $10^{-7}$ M yohimbine. Isometric contractions in response to cumulatively added noradrenaline ($10^{-8}$–$3 \times 10^{-4}$ M) were performed in the absence or presence of antagonists (30 min) after three repetitions of the control curves (Muramatsu et al., 1998).

### 2.3. Rat thoracic aorta and pulmonary artery: $\alpha_{1D}$-adrenoceptors

Ring preparations from the thoracic aorta and pulmonary artery of male rats (Sprague–Dawley, 350–400 g) were mounted in 10-ml organ baths under a resting tension of 1 g in Krebs–Ringer bicarbonate buffer maintained at 37°C and gassed with 95% $O_2$–5% $CO_2$, additionally containing $3 \times 10^{-7}$ M desipramine, $3 \times 10^{-5}$ M corticosterone, $10^{-6}$ M propranolol and $10^{-7}$ M yohimbine. Isometric contractions in response to cumulatively added noradrenaline or buspirone ($3 \times 10^{-9}$–$3 \times 10^{-5}$ M) were performed in the absence or presence of antagonists equilibrated with the tissue for 30 min, when the control response curves had been reproducible and stable after three repetitions (Eltze and Boer, 1992; Eltze et al., 1999).

### 2.4. Field-stimulated rabbit vas deferens: prejunctional $\alpha_2$-adrenoceptors

Male New Zealand white rabbits (2.5–3.0 kg, Charles River) were killed by exsanguination after the animals had been anesthetized with pentobarbital sodium (60 mg/kg, i.v.) and the vasa deferentia were removed. Two prostatic portions of 1 cm in length were folded over a platinum electrode in 10-ml organ baths and connected via a thread to a force-displacement transducer under a resting tension of 0.75 g. A second platinum ring electrode was placed at the top of the bathing fluid for continuous field stimulation by singles pulses (0.05 Hz, 0.5 ms, 30 V). The bathing fluid (mM: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, $KH_2PO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.0) was kept at 31°C and aerated with 95% $O_2$–5% $CO_2$. Neurogenic twitch contractions in response to field stimulation, and their change after cumulative administration of the $\alpha_2$-adrenoceptor agonist, UK 14.304 ($10^{-10}$–$3 \times 10^{-8}$ M), in the absence and presence of AH11110A ($3 \times 10^{-6}$–$3 \times 10^{-5}$ M) equilibrated with the tissue for 30 min, were measured isometrically (Alabaster et al., 1986).

### 2.5. Calculation of antagonist affinities

From the $EC_{50}$ values of the agonist in the presence and absence of different antagonist concentrations, concentration ratios (designated x on the ordinate in the Schild plots) were calculated. Schild plots were constructed to estimate the $pA_2$ value of the antagonist and the slope of regression line from each experimental series, which generally comprised at least three different concentrations (Arunlakshana and Schild, 1959). The slope of the Schild plot is considered an important parameter in that it defines whether or not the data fit the simple competitive model of agonist–antagonist interaction and therefore if the resulting intercept can be considered a chemical term ($pA_2$) to be used to characterize antagonists and receptors in different tissues (Kenakin, 1982, 1987). If the regression was linear and had a slope not significantly different from unity ($P > 0.05$), the regression was recalculated with a constrained slope of unity (Table 1). 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 should be regarded as approximations. In those cases, where the antagonist-induced shift of the agonist curve was greater than expected and accompanied by a depression of the maximal response (AH11110A in rat aorta and pulmonary artery), $pA_2$ values were calculated from unconstrained Schild plots with slopes > 1. All data are presented as means ± S.E.M.

### 2.6. Drugs

1-[Biphenyl-2-yloxy]-4-imino-4-piperidin-1-yl-butan-2-ol HCl (AH11110A; RBI, Cologne, Germany) was dissolved in distilled water to prepare a $10^{-2}$ M stock solution which was further diluted with $H_2O$ directly before the experiments. The compound was also scrutinized for its chemical identity and stability by NMR and mass spectroscopy. 8-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione 2HCl (BMY 7378); 8-[2-(1,4-benzodioxan-2-ylmethylamino)-ethyl]-8-azaspiro[4.5]decane-7,9-dione HCl (MDL 73005EF), spiperone HCl, buspirone HCl, 5-bromo-6[2-imidazolin-2-ylamino]quinazoline (UK 14.304; RBI). Cystazosin was kindly provided by Prof. C. Melchiorre (University of Bologna, Italy). Rec 15/2739 (SB 216469; *N*-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4H-1-benzopyran-8-carboxamide 2HCl) was a gift from Dr. R. Testa (Recordati, Milano,

Table 1
Affinities ($pA_2$) and potencies ($-\log ED_{50}$ mol) of AH11110A in comparison with $\alpha_1$-adrenoceptor subtype-selective antagonists and prazosin at subtype A in rat vas deferens (RVD) and rat kidney (RK), at subtype B in guinea-pig spleen (GPS), mouse spleen (MS) and rabbit aorta (RabA), and at subtype D in rat aorta (RA) and rat pulmonary artery (RPA)

| | Subtype A | | Subtype B | | | Subtype D | |
|---|---|---|---|---|---|---|---|
| Tissue | RVD | RK | GPS | MS | RabA | RA | RPA |
| Agonist | NA ($pA_2$) | NA ($-\log$ mol) | NA ($pA_2$) | NA ($pA_2$) | NA ($pA_2$) | NA ($pA_2$) | Buspirone ($pA_2$) |
| AH11110A | $6.41 \pm 0.09$ (1.03) | $8.00 \pm 0.08$ | $5.94 \pm 0.07$ (1.01) | $5.40 \pm 0.09$[a] (–) | $6.54 \pm 0.05$ (0.93) | $5.47 \pm 0.04$[b] (1.96)[c] | $5.48 \pm 0.03$[b] (1.27)[c] |
| Rec 15/2739 | $10.18 \pm 0.06$ (1.06) | $11.11 \pm 0.12$ | $6.69 \pm 0.07$ (0.98) | $6.99 \pm 0.09$[d] (–) | $7.28 \pm 0.10$ (0.87) | $7.83 \pm 0.11$ (0.74)[c] | $8.07 \pm 0.16$ (0.93) |
| B8805-033 | $8.40 \pm 0.07$ (1.12) | $9.82 \pm 0.13$ | $5.21 \pm 0.08$ (1.05) | $5.34 \pm 0.08$ (0.89) | $5.10 \pm 0.04$ (0.97) | $5.52 \pm 0.12$ (0.88) | $5.45 \pm 0.07$ (1.09) |
| Spiperone | $7.63 \pm 0.03$ (0.93) | $9.54 \pm 0.12$ | $8.05 \pm 0.16$ (0.77)[c] | $8.29 \pm 0.19$ (0.91) | n.d. | $7.82 \pm 0.08$ (0.75)[c] | $8.17 \pm 0.07$ (0.95) |
| BMY 7378 | $6.67 \pm 0.15$ (0.93) | $8.76 \pm 0.19$ | $6.55 \pm 0.18$ (1.02) | $6.76 \pm 0.07$ (0.93) | $6.42 \pm 0.07$ (0.89) | $8.15 \pm 0.16$ (1.00) | $8.00 \pm 0.09$ (1.10) |
| MDL 73005EF | $5.84 \pm 0.08$ (0.93) | $8.08 \pm 0.22$ | $5.88 \pm 0.24$ (0.73)[c] | $6.30 \pm 0.09$ (0.83) | $5.74 \pm 0.09$ (0.68)[c] | $7.23 \pm 0.14$ (1.01) | $7.32 \pm 0.08$ (0.86) |
| Cystazosin | $7.34 \pm 0.06$ (0.96) | $9.63 \pm 0.18$ | $7.16 \pm 0.04$ (0.88) | $7.22 \pm 0.17$ (0.67)[c] | $7.39 \pm 0.07$ (0.81) | $7.89 \pm 0.08$ (0.93) | $8.06 \pm 0.08$ (0.97) |
| Prazosin | $8.90 \pm 0.13$ (0.91) | $10.35 \pm 0.17$ | $9.07 \pm 0.09$ (0.99) | $8.98 \pm 0.11$ (0.88) | 8.82[e] | $8.85 \pm 0.09$ (0.90) | $9.45 \pm 0.12$ (1.08) |

With the exception of AH11110A at $\alpha_{1D}$-adrenoceptors in RA and RPA (Schild plot slopes significantly greater than 1.00), all other $pA_2$ values (with Schild plot slopes in parentheses) were calculated from constrained regression lines for competitive antagonism. The results are presented as means ± S.E.M. of $n = 6$–7 for rat kidney and $n = 12$–16 for $pA_2$ determinations for each drug in the different tissues. Most data for the reference antagonists on rat vas deferens, kidney and aorta, guinea-pig and mouse spleen were taken from Eltze and Boer (1992), Eltze (1994, 1996b), Eltze et al. (1999).
n.d., not determined.

[a] $pA_2$ value determined at the single concentration of $10^{-5}$ M.
[b] $pA_2$ calculated at a slope as indicated.
[c] Slope significantly different from unity ($P < 0.05$).
[d] $pA_2$ value determined at the single concentration of $10^{-6}$ M.
[e] Value taken from Leonardi et al. (1997).

Italy). (±)-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) (Byk Gulden, Konstanz, Germany). All other drugs were purchased from Sigma (Munich, Germany).

## 3. Results

### *3.1. $\alpha_{1A}$-adrenoceptors: rat vas deferens and rat perfused kidney*

When AH11110A ($3\times10^{-7}$–$10^{-5}$ M) was equilibrated with rat vas deferens for 20 min, it caused parallel shifts to the right of the noradrenaline concentration–response curve, indicating mainly competitive antagonism at $\alpha_{1A}$-adrenoceptors in this tissue. However, in the presence of AH11110A there was an increase in the maximum response of 20–35% relative to the control concentration–response curve to noradrenaline, although the magnitude of this change did not appear to be dependent on the antagonist concentration (Fig. 2, top). In spite of the increase in the maximum contraction, a Schild plot was constructed which gave a $pA_2$ value of 6.41 from the constrained regression line ($pA_2 = 6.38 \pm 0.07$ at a slope $= 1.03 \pm 0.07$; not significantly different from 1.00, $P > 0.05$) (Fig. 2, middle). The reference antagonists, Rec 15/2739, B8805-033, spiperone, BMY 7378, MDL 73005EF and cystazosin, competitively antagonized these contractions, yielding $pA_2$ values from constrained Schild plots of 10.18, 8.40, 7.63, 6.67, 5.84 and 7.34, respectively (Fig. 2, middle; Table 1).

The increase in maximum response to noradrenaline by AH11110A was further investigated by studying its effect on $K^+$ contractions. KCl (15–75 mM) produced concentration-dependent and reproducible isotonic contractions of rat vas deferens, which, in the presence of $10^{-7}$–$3\times10^{-6}$ M of AH11110A, were increased by a similar amount (20–30%) as those evoked by noradrenaline in this tissue (Fig. 3).

During vasoconstriction evoked in rat perfused kidney by noradrenaline ($6\times10^{-7}$ M), injections of increasing doses of AH11110A ($10^{-10}$–$3\times10^{-8}$ mol) caused a dose-dependent and reversible increase in perfusion flow, however, at $5\times10^{-8}$ and $10^{-7}$ mol this effect was weaker ($63\pm9$% and $46\pm6$%, respectively) than the maximal vasodilation ($67\pm5$%) elicited by $3\times10^{-8}$ mol of AH11110A, resulting in a bell-shaped dose–response curve (Fig. 2, bottom). The $-\log ED_{50}$ (mol) value for a half-maximal blocking effect by AH11110A was 8.00 (Table 1).

In both preparations, Rec 15/2739 and B8805-033 were the most potent antagonists of noradrenaline; however, the weakest antagonists, AH11110A and MDL 73005EF, showed a slightly different rank order of affinity in rat vas deferens (AH11110A > MDL 73005EF) compared to their equal potency in rat kidney (AH11110A = MDL 73005EF).

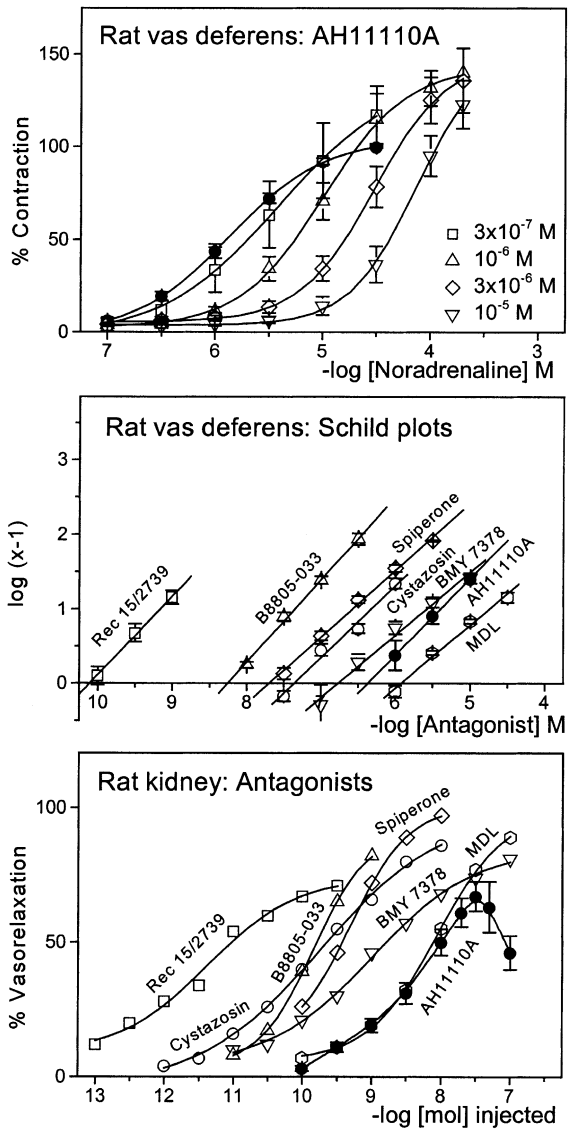



Fig. 2. Top: Concentration–response curves of noradrenaline to evoke contraction of rat vas deferens in the absence (filled circles) or presence of increasing concentrations of AH11110A (open symbols) equilibrated with the tissue for 20 min (mean ± S.E.M., $n = 12$ for the control, $n = 4$–9 in the presence of each concentration of AH11110A). Middle: Schild plots for the antagonism by AH11110A and reference antagonists against noradrenaline-induced contractions in rat vas deferens (mean ± S.E.M. of $n = 4$–9). Bottom: Dose–response curves for inhibition by AH11110A and reference antagonists of renal vasoconstriction induced by continuous presence of $6\times10^{-7}$ M noradrenaline in perfused rat kidney (mean ± S.E.M. of $n = 6$–7; S.E.M. < 10% not shown for the reference compounds). Abbreviation: MDL = MDL 73005EF.

### *3.2. $\alpha_{1B}$-adrenoceptors: guinea-pig spleen, mouse spleen and rabbit aorta*

In isolated spleen strips from guinea-pig, AH11110A at concentrations of $3\times10^{-6}$ and $10^{-5}$ M, caused competi-

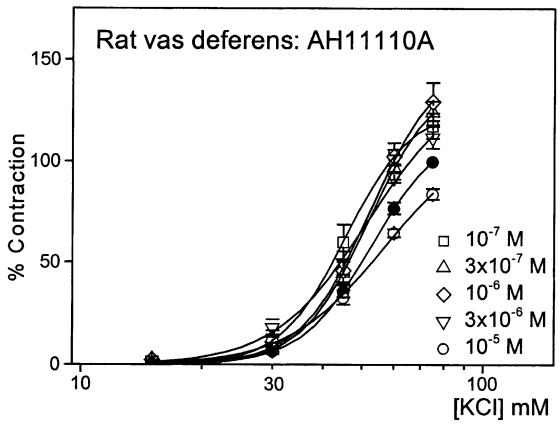



Fig. 3. Concentration–response curves of KCl to evoke contraction of rat vas deferens in the absence (filled circles) or presence of increasing concentrations of AH11110A (open symbols) equilibrated with the tissue for 20 min (mean ± S.E.M., $n = 16$ for the control, $n = 4$–5 in the presence of each concentration of AH11110A).

tive and concentration-related antagonism against tissue contraction evoked by noradrenaline, whereas at $3 \times 10^{-5}$ M the shift of the agonist curve was weaker than expected for competitive antagonism (Fig. 4, top). The $pA_2$ value calculated from a constrained Schild plot including the concentrations of $3 \times 10^{-6}$ and $10^{-5}$ M of AH11110A, amounted to 5.94 ($pA_2 = 5.93 \pm 0.07$ at a slope = $1.01 \pm 0.07$, not significantly different from 1.00, $P > 0.05$) (Fig. 4, top), but was lower ($pA_2 = 5.56 \pm 0.10$) using the single concentration of $3 \times 10^{-5}$ M. In mouse spleen, $3 \times 10^{-6}$–$3 \times 10^{-5}$ M of AH11110A caused weak rightward displacements of the noradrenaline concentration–response curve, however, this shift was not concentration-related (Fig. 4, bottom). Using the concentration ratio evoked by $10^{-5}$ M of AH11110A, a $pA_2$ estimate of 5.40 was obtained. The reference antagonists investigated caused parallel shifts to the right of the noradrenaline concentration–response curves in guinea-pig and mouse spleen, indicating competitive antagonism (not shown). Schild plots for the reference antagonists investigated in these preparations (Fig. 4, top and bottom) were linear and, with the exception of spiperone and MDL 73005EF in guinea-pig spleen and Rec 15/2739 in mouse spleen, yielded slopes of regression not significantly different from unity ($P > 0.05$; Table 1).

In rabbit thoracic aorta, AH11110A ($3 \times 10^{-7}$–$10^{-5}$ M) behaved as a competitive antagonist against tissue contraction in response to noradrenaline with no depression of the maximum response (Fig. 5, top), yielding a

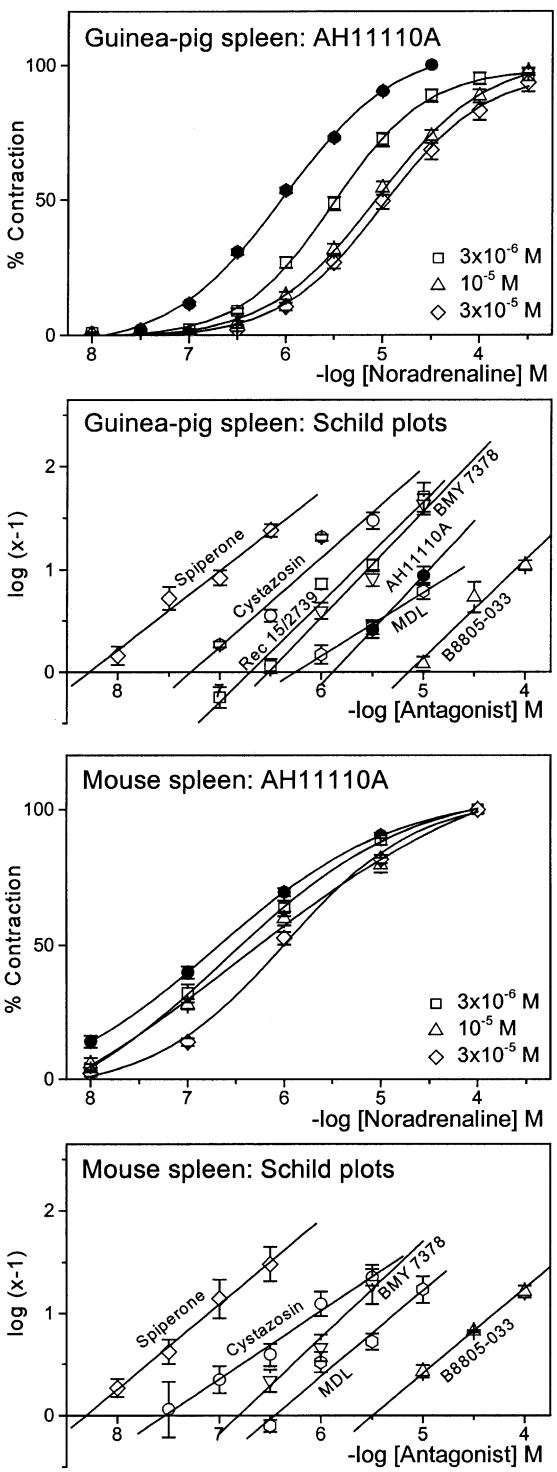



Fig. 4. Top: Concentration–response curves of noradrenaline to evoke contraction of guinea-pig spleen in the absence (filled circles) or presence of increasing concentrations of AH11110A (open symbols) equilibrated with the tissue for 30 min (mean ± S.E.M., $n = 18$ for the control, $n = 9$ in the presence of each concentration of AH11110A), and Schild plots for the antagonism by AH11110A and reference antagonists against contractions evoked by noradrenaline in guinea-pig spleen (mean ± S.E.M., $n = 9$–12). Bottom: Concentration–response curves of noradrenaline to evoke contraction of mouse spleen in the absence (filled circles) or presence of increasing concentrations of AH11110A (open symbols) equilibrated with the tissue for 30 min (mean ± S.E.M., $n = 18$ for the control, $n = 9$ in the presence of each concentration of AH11110A), and Schild plots for the antagonism by reference antagonists against contractions evoked by noradrenaline in mouse spleen (mean ± S.E.M., $n = 9$–12). Abbreviation: MDL = MDL 73005EF.

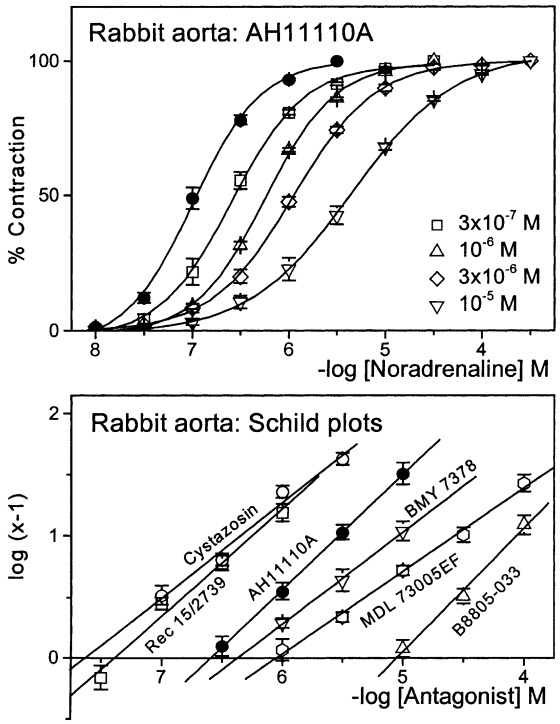



Fig. 5. Top: Concentration–response curves of noradrenaline to evoke contraction of rabbit aorta in the absence (filled circles) or presence of increasing concentrations of AH11110A (open symbols) equilibrated with the tissue for 30 min (mean ± S.E.M., $n = 18$ for the control, $n = 9$ in the presence of each concentration of AH11110A). Bottom: Schild plots for the antagonism by AH11110A and reference antagonists against contractions evoked by noradrenaline in rabbit aorta (mean ± S.E.M., $n = 9$–12).

$pA_2$ value of 6.54 ± 0.05 ($pA_2 = 6.61$ at a slope of 0.93 ± 0.03, not significantly different from 1.00, $P > 0.05$) (Fig. 5, bottom; Table 1). Whereas this value differed at least 4-fold from those calculated for AH11110A at guinea-pig and mouse splenic $\alpha_{1B}$-adrenoceptors (5.94 and 5.40, respectively), the affinities determined from linear and constrained Schild plots in rabbit aorta experiments for the A/B-subtype discriminating Rec 15/2739 and B8805-033, $pA_2 = 7.28$ and 5.10, respectively, and D/B-subtype discriminating BMY 7378, MDL 73005EF and cystazosin, $pA_2 = 6.42$, 5.74 and 7.39, respectively, were consistent with those at guinea-pig and mouse splenic $\alpha_{1B}$-adrenoceptors (Fig. 5, bottom; Table 1), thereby confirming the suggestion of Muramatsu et al. (1998) of $\alpha_{1B}$-adrenoceptors mediating the noradrenaline-induced contractions in rabbit aorta.

### 3.3. $\alpha_{1D}$-adrenoceptors: rat thoracic aorta and pulmonary artery

In rat thoracic aorta, AH11110A ($3 \times 10^{-6}$–$3 \times 10^{-5}$ M) did not appear to be a competitive antagonist against

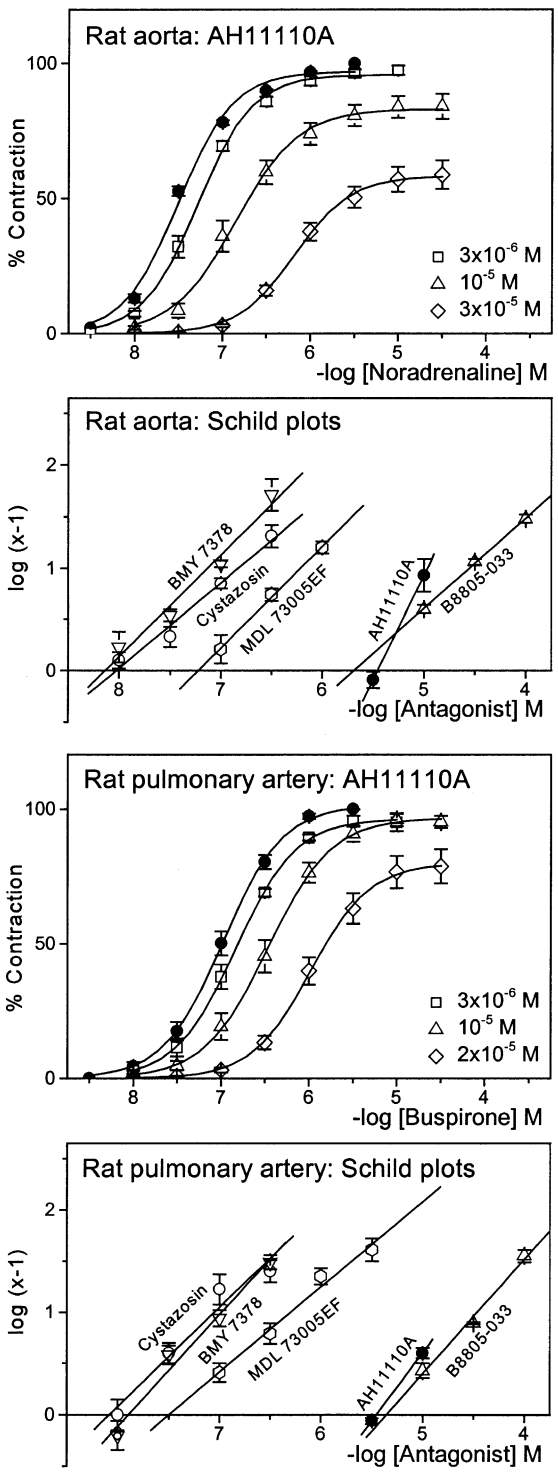



Fig. 6. Top: Concentration–response curves of noradrenaline to evoke contraction of rat aorta in the absence (filled circles) or presence of increasing concentrations of AH11110A (open symbols) equilibrated with the tissue for 30 min (mean ± S.E.M., $n = 18$ for the control, $n = 9$ in the presence of each concentration of AH11110A), and Schild plots for the antagonism by AH11110A and reference antagonists against contractions evoked by noradrenaline in rat aorta (mean ± S.E.M., $n = 9$–12). Bottom: Concentration–response curves of buspirone to evoke contraction of rat pulmonary artery in the absence (filled circles) or presence of increasing concentrations of AH11110A (open symbols) equilibrated with the tissue for 30 min (mean ± S.E.M., $n = 18$ for the control, $n = 9$ in the presence of each concentration of AH11110A), and Schild plots for the antagonism by AH11110A and reference antagonists against contractions evoked by buspirone in rat pulmonary artery (mean ± S.E.M., $n = 9$–12).

noradrenaline-evoked smooth muscle contraction, as the shifts of the agonist concentration–response curves were greater than expected and significantly depressed by more than 40% at $3 \times 10^{-5}$ M of AH11110A (Fig. 6, top). A $pA_2$ of 5.47 (at a slope = $1.96 \pm 0.04$, significantly different from 1.00, $P < 0.001$) was obtained using $3 \times 10^{-6}$ and $10^{-5}$ M of AH11110A, two concentrations yet appearing to cause a reasonably competitive antagonism (Fig. 6, top; Table 1). When buspirone was used in rat pulmonary artery to selectively stimulate $\alpha_{1D}$-adrenoceptors in this tissue (Eltze et al., 1999), AH11110A at $3 \times 10^{-6}$ and $10^{-5}$ M caused parallel shifts to the right of the agonist curve, whereas concentrations higher than $2 \times 10^{-5}$ M, which already depressed the maximal response to buspirone, were not investigated (Fig. 6, bottom). By use of $3 \times 10^{-6}$ and $10^{-5}$ M of AH11110A for constructing the Schild plot, a $pA_2$ value of 5.48 (at a slope = $1.27 \pm 0.12$, significantly different from 1.00, $P < 0.05$) was calculated (Fig. 6, bottom), which corresponds to that obtained in rat aorta against noradrenaline (Table 1). Insufficient equilibrium time was not the cause of the steep regression lines observed for AH11110A in both preparations, since longer incubation times (up to 1 h) did not change their steepness and resulting $pA_2$ values (not shown). Thus, both in rat aorta and pulmonary artery the inhibition observed with AH11110A was inconsistent with competitive antagonism at $\alpha_{1D}$-adrenoceptors. In contrast, the regression lines for all reference antagonists investigated in both tissues, with the exception of Rec 15/2739 and spiperone in rat aorta, had slopes not significantly different from unity ($P > 0.05$) (Fig. 6, bottom; Table 1).

### *3.4. Prejunctional $\alpha_2$-adrenoceptors: field-stimulated rabbit vas deferens*

The antagonism by AH11110A on prejunctional $\alpha_2$-adrenoceptors was studied in the field-stimulated rabbit vas deferens using UK 14.304 as a selective $\alpha_2$-adrenoceptor agonist. In this tissue, the concentration–response curve for UK 14.304 was shifted to the right in a parallel and concentration-dependent manner by AH11110A ($3 \times 10^{-6}$ and $10^{-5}$ M), however, the highest concentration tested ($3 \times 10^{-5}$ M) also slightly depressed the maximum response of the agonist (Fig. 7). Within the concentration range of $3 \times 10^{-6}$–$3 \times 10^{-5}$ M AH11110A, the slope of regression ($0.66 \pm 0.11$; $n = 3$) was significantly lower than unity ($P < 0.05$) and therefore, the inhibition by AH11110A must be regarded inconsistent with competitive antagonism. A $pA_2$ estimate of 5.44 was obtained for AH11110A with the lowest concentration tested ($3 \times 10^{-6}$ M). As a reference standard, we found yohimbine ($3 \times 10^{-7}$–$10^{-5}$ M) to be a competitive antagonist ($pA_2 = 6.71$, slope = 1.08) at $\alpha_2$-adrenoceptors in this preparation (not shown).

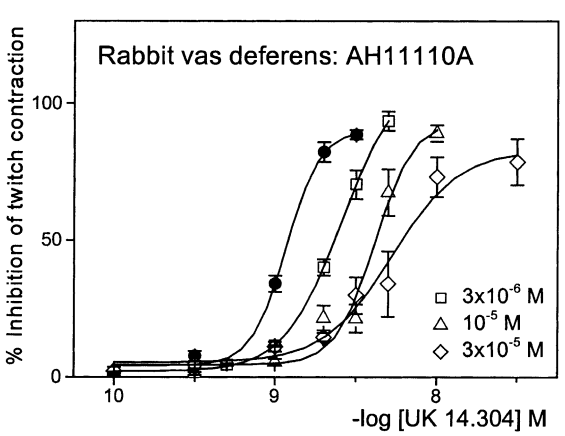



Fig. 7. Concentration–response curves for UK 14.304 to inhibit field stimulation-evoked contractions in rabbit vas deferens in the absence (filled circles) and presence of AH111110A (open symbols) equilibrated with the tissues for 30 min (mean ± S.E.M., $n = 7$–12).

## 4. Discussion

The $\alpha_1$-adrenoceptor antagonist, AH11110A, has been reported to have a roughly 10- and 20-fold higher affinity at the native or cloned subtype B(b) (range in $pK_i$: 7.10–7.73) over subtypes A(a) (range in $pK_i$: 5.59–7.02) and D(d) (range in $pK_i$: 5.68–6.42), respectively, as deduced from radioligand binding studies in tissues from different animal (rat, hamster, bovine) and human sources (King et al., 1994; Giardina et al., 1996; Saussy et al., 1996), and is cited in a number of recent reviews for its presumed usefulness for $\alpha_1$-adrenoceptor characterization (Ruffolo et al., 1995; Hancock, 1996). With the exception of its affinity at rat aortic $\alpha_{1D}$-adrenoceptors ($pA_2 = 6.29$; King et al., 1994), no other functional affinity data of the compound at $\alpha_1$-adrenoceptor subtypes A, B and D have been published to date. Nevertheless, due to its favourable binding profile the compound is now recommended as the only selective $\alpha_{1B}$-adrenoceptor antagonist (see Introduction). In the present study, we have tried to replicate the findings for the $\alpha_{1B}$-adrenoceptor selectivity of AH11110A by using a number of reliable functional experiments.

The reference antagonists, Rec 15/2739 and B8805-033, were confirmed as highly selective for $\alpha_{1A}$-adrenoceptors in rat vas deferens and rat kidney, as opposed to $\alpha_{1B}$-adrenoceptors in guinea-pig, mouse spleen and rabbit aorta, and $\alpha_{1D}$-adrenoceptors in rat aorta and pulmonary artery ($\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$, and $\alpha_{1A} > \alpha_{1D} = \alpha_{1B}$, respectively). Their functional affinity profiles are consistent with respective binding profiles at native and recombinant animal and human $\alpha_1$-adrenoceptor subtypes A(a), B(b) and D(d) (Testa et al., 1997; Eltze et al., 1999). B8805-033 exceeds Rec 15/2739 in having a 1000-fold selectivity for subtype A over both subtypes B and D, and thus emerges as a most interesting ligand selective for $\alpha_{1A}$-adrenoceptors (Eltze et al., 1996, 1999). BMY 7378 and MDL 73005EF displayed an approximately 30- and 20-fold se-

lectivity, respectively, for $\alpha_{1D}$-adrenoceptors located on rat aorta and pulmonary artery as opposed to tissues endowed with $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptors ($\alpha_{1D} > \alpha_{1A} = \alpha_{1B}$). Cystazosin qualitatively resembles these compounds, but exhibited a somewhat weaker selectivity (< 10-fold) for subtype D ($pA_2 = 7.89$ and 8.08) over both subtypes A and B than that previously found for this drug (10-fold), due to a somewhat higher affinity ($pA_2 = 8.54$) initially determined on rat aorta (Bolognesi et al., 1998). Selectivity of spiperone was only evident for subtype B in guinea-pig and mouse spleen over subtype A in rat vas deferens (3-fold), but not detectable when comparing its affinity at subtype B with subtype D in rat aorta and pulmonary artery. However, the failure of spiperone for discrimination of subtypes B and D, has also previously been found in binding studies at native or cloned $\alpha_1$-adrenoceptor subtypes (Blue et al., 1995; Giardina et al., 1995; Kenny et al. 1995; Schwinn et al., 1995).

Surprisingly, despite the restriction that, in most tissues the antagonism exerted by AH11110A was non-competitive in nature, which renders exact calculation of true affinities more difficult, the affinities of AH11110A at subtype A in rat vas deferens (mainly competitive with $pA_2 = 6.41$), at subtype B in guinea-pig spleen (competitive at $3 \times 10^{-6}$ and $10^{-5}$ M with $pA_2 = 5.94$) and mouse spleen (not concentration-dependent with $pA_2 = 5.40$) and rabbit aorta (competitive with $pA_2 = 6.54$) and at subtype D in rat aorta and pulmonary artery (non-competitive with $pA_2 = 5.47$ and 5.48, respectively), were nearly in the same order of magnitude and reveal no selectivity of the compound for any of these subtypes. Presently, there is no apparent explanation available for the discrepancy observed between the affinities of AH11110A derived from earlier binding studies at native $\alpha_{1B}$- and cloned $\alpha_{1b}$-adrenoceptors, yielding relatively high $pK_i$ values between 7.10 and 7.73 (King et al., 1994; Giardina et al., 1996; Saussy et al., 1996), and values from our functional experiments at guinea-pig and mouse splenic $\alpha_{1B}$-adrenoceptors. However, the high affinity of spiperone ($pA_2 = 8.05$ and 8.29, respectively) contrasting to the low affinity of B8805-033 ($pA_2 = 5.21$ and 5.34, respectively), characterize the splenic tissue of both species as reliable functional assays for $\alpha_{1B}$-adrenoceptors (Eltze, 1994, 1996b). Moreover, the proposal for $\alpha_{1B}$-adrenoceptor stimulation in the rabbit aorta, when noradrenaline is used as the agonist to evoke contraction (Muramatsu et al., 1998), was confirmed in our study revealing consistent $pA_2$ values for the $\alpha_1$-adrenoceptor subtype A/B-discriminating antagonists, Rec 15/2739 and B8805-033, and the subtype D/B-discriminating antagonists, BMY 7378, MDL 73005EF and cystazosin, between rabbit aortic and both guinea-pig and mouse splenic $\alpha_{1B}$-adrenoceptors. Particularly, the low affinities displayed by B8805-033 ($pA_2 = 5.10$) and BMY 7378 ($pA_2 = 6.42$) against noradrenaline-evoked contractions in rabbit aorta, in contrast to their 2000- and 40-fold higher affinities at $\alpha_{1A}$- and $\alpha_{1D}$-adrenoceptors, respectively, make these receptors unlikely candidates to be involved in rabbit aortic contraction. There was no discontinuity seen in the Schild plots, which also excludes the possible involvement of more than one receptor in the contractile response to noradrenaline in this tissue. Only in rabbit aorta, a competitive antagonism by AH11110A over a greater concentration range (factor 30) could be achieved, however, the $pA_2$ value obtained (6.54) was higher than those at respective splenic $\alpha_{1B}$-adrenoceptors ($pA_2 < 6.0$), the possible reason for this discrepancy is discussed below.

Similar differences in affinities of antagonists at $\alpha_{1B}$-adrenoceptors have been observed for, e.g. risperidone (Sleight et al., 1993) and cyclazosin or its (+)-enantiomer (Giardina et al., 1995, 1996), previously characterized as $\alpha_{1B}$-adrenoceptor selective compounds by means of binding studies, but later shown to be devoid of this property using functional experiments (Eltze, 1996a; Stam et al., 1998). Conversely, binding assays with human cloned $\alpha_1$-adrenoceptor subtypes a, b and d performed with a series of quinazoline compounds including cystazosin, yielded uniform affinity values which were not in accordance with their selectivity behaviour obtained in functional experiments at respective native subtypes A, B and D (Bolognesi et al., 1998). Why the functional affinity profiles of those compounds differ from those obtained in radioligand binding on membranes is an as yet unresolved observation requiring further investigation.

On the other hand, the affinity obtained for AH11110A at rat vas deferens $\alpha_{1A}$-adrenoceptors ($pA_2 = 6.41$) was consistent with the drug's average binding affinity at the native and recombinant $\alpha_1$-adrenoceptor subtype A(a) ($pK_i = 5.59–7.02$; Giardina et al., 1996; Saussy et al., 1996). However, the affinities of AH11110A at rat aortic and pulmonary artery $\alpha_{1D}$-adrenoceptors ($pA_2 = 5.47$ and 5.48, respectively) were somewhat lower than its range of binding affinities previously obtained at subtype D(d) ($pK_i = 5.68–6.42$; Giardina et al., 1996; Saussy et al., 1996), the discrepancy of which might be due to an underestimation of its affinities in rat aorta and pulmonary artery because of the steep and unconstrained Schild plots used for their calculation. Theoretically, constraining the slopes to unity would result in $pA_2$ values near 6.0 in both cases, however, this method is strictly not applicable because of slopes significantly greater than unity. In earlier functional studies on rat aortic $\alpha_{1D}$-adrenoceptors, a $pA_2$ value of 6.29 has been found for AH11110A, but it is unknown which single concentration of the compound has been used for its determination (King et al., 1994; Saussy et al., 1996).

At prejunctional $\alpha_2$-adrenoceptors on sympathetic nerve endings in rabbit vas deferens (Alabaster et al., 1986), AH11110A displayed an affinity value ($pA_2 = 5.44$), which is consistent with its binding affinity ($pK_i = 5.54$) previously found at rat cerebral $\alpha_2$-adrenoceptors (Giardina et al., 1996). In an alternative model for prejunctional $\alpha_2$-adrenoceptors, namely the electrically stimulated longitudi-

nal muscle strip of the guinea-pig ileum (Wikberg, 1978), AH11110A ($10^{-5}$–$3 \times 10^{-5}$ M) caused concentration-dependent inhibition of twitch contractions during equilibration ($IC_{50} = 2.5 \times 10^{-5}$ M), and therefore could not be investigated for its antagonism at $\alpha_2$-adrenoceptors on cholinergic nerve endings in this tissue (not shown). Similarly, AH11110A ($3 \times 10^{-6}$–$3 \times 10^{-5}$ M) caused non-competitive inhibition of guinea-pig ileum contractions (approximate $IC_{50} = 10^{-5}$ M) evoked by exogeneous acetylcholine, but not of those evoked by histamine, and caused no relaxation of the spontaneously contracted guinea-pig trachea up to $3 \times 10^{-5}$ M (not shown), thus rendering blockade of muscarinic receptors and not a smooth muscle relaxing property responsible for the twitch depressant effect observed for AH11110A in the field-stimulated guinea-pig ileum preparation.

The anomalous effects evoked by AH11110A in most of the tissues used, inconsistent with simple competitive interaction with $\alpha_1$-adrenoceptor subtypes, deserves consideration. First, in rat vas deferens, AH11110A caused mainly competitive antagonism of noradrenaline, but concomitantly potentiated its maximum response, although cocaine was present in the nutrient solution. Theoretically, this could be due to inhibition of other mechanism(s) than blockade of neuronal uptake for removing noradrenaline from the receptor compartment and/or a smooth muscle sensitizing effect in rat vas deferens, a similar phenomenon previously observed, e.g. with yohimbine in this tissue (Jurkiewicz and Jurkiewicz, 1976; Kenakin, 1982). However, the increase in maximum contraction evoked by both noradrenaline and KCl in rat vas deferens by AH11110A in the same concentration range ($3 \times 10^{-7}$–$3 \times 10^{-6}$ M) and by the same amount (20–30%), makes uptake blockade unlikely, but rather suggests that AH11110A enhances the general contractility of the tissue. This property could also explain the finding that AH11110A, at threshold concentrations of $3 \times 10^{-6}$ M, enhances the field stimulation-induced twitch contractions of rat vas deferens (30 V, 3 ms at 0.1 Hz) by 20–30% (not shown). Second, in perfused rat kidney as the alternative $\alpha_{1A}$-adrenoceptor model, AH11110A evoked vasodilation (67% of maximum) that was lower than that of most reference antagonists (> 80%) and produced a bell-shaped dose–response curve, pointing to a self-cancelling mechanism of $\alpha_{1A}$-adrenoceptor blockade at higher doses of AH11110A. This would make AH11110A less effective as an antagonist in this tissue and could explain, why AH11110A is only equipotent to MDL 73005EF in rat kidney, but 4-fold more potent than MDL 73005EF in rat vas deferens. Third, in guinea-pig and mouse splenic strips, no concentration-related antagonism by AH11110A at concentrations higher than $10^{-5}$ M could be achieved, which cannot be ascribed to blockade of neuronal and extra-neuronal uptake or of β-adrenoceptors in these tissues, since the nutrient solution contained desipramine, corticosterone and propranolol. Moreover, it cannot be excluded that this self-cancellation by AH11110A in both splenic preparations might already been active at lower concentration ($< 3 \times 10^{-6}$ M) than those found to be inhibitory and used for calculation of $pA_2$ values in these tissues ($> 3 \times 10^{-6}$ M), since in rabbit aorta as the alternative $\alpha_{1B}$-adrenoceptor model, AH11110A, already at threshold concentrations of $3 \times 10^{-7}$ M, acted as a simple competitive antagonist and produced a significantly higher value ($pA_2 = 6.54$) than those derived from guinea-pig and mouse spleen experiments ($pA_2 = 5.94$ and 5.40, respectively). This suggests, that the former value may be regarded a better estimate for its "true" functional affinity at $\alpha_{1B}$-adrenoceptors, nevertheless, this value is not in accordance with the relatively high affinity (range in $pK_i$: 7.10–7.73) reported for AH11110A from binding experiments at native or cloned $\alpha_{1B}$-adrenoceptors (Giardina et al., 1996; Saussy et al., 1996). Finally, the steep slopes of the Schild plots for AH11110A in both rat aorta (1.96) and pulmonary artery (1.27), in contrast to most other antagonists investigated, were unlikely to be due to lack of time to reach equilibrium, since a longer incubation time (1 h) did not approach its slopes near unity, but possibly are indicative of chemical interference and/or saturation of antagonist removal (for review, see Kenakin, 1987). As a result, these steep regression lines reveal non-compliance of the Schild equation and preclude valid determination of "true" affinities of AH11110A at $\alpha_{1D}$-adrenoceptors in rat aorta and pulmonary artery, and therefore both $pA_2$ values obtained may be considered to be underestimates. Also this phenomenon was not further analysed. Since in the majority of tissues chosen to determine its affinity at different $\alpha_1$-adrenoceptors, the antagonism by AH11110A was not simply competitive but superposed by a number of as yet unresolved effects, the compound may generally complicate further smooth muscle functional studies intended to be undertaken for $\alpha_1$-adrenoceptor subtype characterization.

In conclusion, data from our present functional experiments confirmed the selectivity of Rec 15/2739 and B8805-033 for the A subtype, of BMY 7378, MDL 73005EF and cystazosin for the D subtype, and a weak selectivity of spiperone for the B over the A subtype, but characterized AH11110A as a low affinity, unselective and in most cases non-competitive antagonist with respect to these $\alpha_1$-adrenoceptor subtypes. Additionally, we found AH11110A to be almost equipotent at $\alpha_1$- and $\alpha_2$-adrenoceptors. As a result, the claimed usefulness of AH11110A for $\alpha_{1B}$-adrenoceptor subtype characterization, at least by means of functional experiments, appears unjustified.

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