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

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1 The α_1 -adrenoceptor subtype mediating contraction of the rat epididymal vas deferens and rat spleen has been investigated by use of α_1 -adrenoceptor antagonists that have shown selectivity between the different cloned receptor subtypes.

2 In the rat epididymal vas deferens the potency of noradrenaline and phenylephrine was increased in the presence of neuronal and extra-neuronal uptake blockers, cocaine and β -oestradiol, but these did not alter that of methoxamine. The order of potency of the agonists in the presence or absence of uptake blockade was noradrenaline > phenylephrine > methoxamine. In the rat spleen the potency of these agonists was not altered in the presence of cocaine and β -oestradiol, and their order of potency was the same as in the vas deferens.

3 The non subtype selective α_1 -adrenoceptor antagonist prazosin (up to 1×10^{-7} M) was found to antagonize contractions to noradrenaline in the vas deferens competitively (pA_2 9.2), but only in a non competitive manner in the spleen. Contractions to phenylephrine in the spleen however were competitively antagonized by prazosin (up to 1×10^{-7} M) with a pA_2 of 9.2. This suggests that there is an α_1 - and a non α_1 -adrenoceptor response to noradrenaline in the rat spleen.

4 Pretreatment with chlorethylclonidine (10^{-4} M for 30 min) did not alter the noradrenaline contractions in the vas deferens, but contractions to noradrenaline and phenylephrine in the spleen were shifted 30 and 300 fold to the right of the control curve, respectively. This suggests that only the contractions in the spleen were mediated by α_{1B} -adrenoceptors.

5 The noradrenaline contractions in the vas deferens were competitively antagonized by WB 4101 (pA_2 9.6), 5-methyl-urapidil (pA_2 8.7), phentolamine (pA_2 8.3), benoxathian (pA_2 9.4), spiperone (pA_2 7.5), indoramin (pA_2 8.4) and BMY 7378 (pA_2 6.7), consistent with the affinities of these antagonists in binding studies on tissue α_{1A} -adrenoceptors. These values correlated best with their published affinities on the expressed α_{1C} -adrenoceptor clone and poorly with those at either the expressed α_{1B} - or α_{1D} -adrenoceptor clones. Therefore the classical α_{1A} -adrenoceptor appears to be the same as the expressed α_{1C} -adrenoceptor clone.

6 The phenylephrine contractions in the spleen were competitively antagonized by WB 4101 (pA_2 8.1), 5-methyl-urapidil (pA_2 7.1), phentolamine (pA_2 7.3), benoxathian (pA_2 7.4), spiperone (pA_2 7.9), indoramin (pA_2 7.5) and BMY 7378 (pA_2 7.4), consistent with the affinities of these antagonists in binding studies on tissue α_{1B} -adrenoceptors. The pA_2 values correlated best with the published affinities of these compounds on the expressed α_{1B} -adrenoceptor clone and poorly with those at either the expressed α_{1D} - or α_{1C} -adrenoceptor clones. Therefore the α_{1B} -adrenoceptor appears to be the same as the expressed α_{1B} -adrenoceptor clone.

7 The results provide pharmacological evidence that the α_1 -adrenoceptor mediating noradrenaline contractions in the epididymal portion of the rat vas deferens is the α_{1A} -(α_{1C}) subtype and that contractions to phenylephrine in the rat spleen are mediated by the α_{1B} -subtype.

Keywords: α_1 -Adrenoceptor subtypes; rat vas deferens; rat spleen; WB 4101; 5-methyl-urapidil; spiperone; benoxathian; indoramin; BMY 7378; chlorethylclonidine

Introduction

There has been evidence for the existence of subtypes of the α_1 -adrenoceptor for several years. High and low affinity site binding of WB 4101 for α_1 -adrenoceptors in rat brain was found by Morrow & Creese (1986). Functional and binding studies showed that chlorethylclonidine alkylated α_1 -adrenoceptors in certain tissues to a much greater extent than in others, and these chlorethylclonidine-sensitive receptors had a lower affinity for the competitive α_1 -adrenoceptor antagonist, WB 4101 as compared with the chlorethylclonidine-insensitive receptors (Han *et al.*, 1987a,b). Receptors with a higher affinity for WB4101 were termed the α_{1A} subtype while those being

more sensitive to alkylation by chlorethylclonidine were termed the α_{1B} subtype. Functionally defined receptors are now denoted by upper case and cloned receptors by lower case letters (Bylund *et al.*, 1994).

Two α_1 -adrenoceptors have now been cloned from a rat cerebral cortex cDNA library (using a probe based on a hamster α_{1B} -adrenoceptor, Cotecchia *et al.*, 1988), which appeared to have pharmacological profiles in cell lines where they have been expressed corresponding to the α_{1A} and α_{1B} subtypes (Lomasney *et al.*, 1991). However a near identical clone to the one thought to correspond with the α_{1A} subtype was cloned by Perez *et al.* (1991). When expressed in cell lines this clone had a different pharmacological profile from the α_{1A} subtype as it was found to be partially chlorethylclonidine-sensitive and had a lower affinity for 5-methyl urapidil compared with tissue α_{1A} -

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adrenoceptors. It was therefore termed the α_{1d} subtype and the two near identical clones were then sometimes referred to as the $\alpha_{1a/d}$ subtype. Schwinn & Lomasney (1992) subsequently agreed with Perez *et al.* (1991), showing that their clone when expressed in cell lines also had a low affinity for 5-methyl urapidil and benoxathian.

A third α_1 -adrenoceptor has also been cloned, initially from a bovine brain cDNA library which when expressed had a similar pharmacology to the α_{1A} subtype except that it was also partially chlorethylclonidine-sensitive (Schwinn *et al.*, 1990) and was not thought to be expressed in the rat as it could not be detected by Northern blot analysis (Schwinn *et al.*, 1991). This receptor was termed α_{1c} (Schwinn *et al.*, 1990). A homologous clone of this subtype has now been found in the rat (Laz *et al.*, 1993; Perez *et al.*, 1994) and it has been suggested by Laz *et al.* (1993) and Forray *et al.* (1994a) that the rat α_{1c} clone is quite insensitive to chlorethylclonidine compared with the bovine clone. Recently using RNase protection assays the α_{1c} clone has been found to be expressed in several rat tissues (Perez *et al.*, 1994; Rokosh *et al.*, 1994).

The affinities of several different antagonists have now been measured in binding studies using membranes from cells transfected with the cDNA for the three subtypes (Faure *et al.*, 1994; Forray *et al.*, 1994b; Kenny *et al.*, 1994a,b; Saussy *et al.*, 1994; Testa *et al.*, 1994; Goetz *et al.*, 1995). While prazosin was shown to be non selective, WB 4101, 5-methyl urapidil, phentolamine, benoxathian, spiperone, indoramin and BMY 7378 all showed some degree of selectivity. In particular BMY 7378 has recently been shown to be very selective for the α_{1d} subtype (Saussy *et al.*, 1994; Goetz *et al.*, 1995).

Using RNase protection assays the α_{1b} , α_{1c} and α_{1d} clones have been found to be expressed in the rat vas deferens, while the α_{1b} and α_{1d} clones have been found to be expressed in the rat spleen (Perez *et al.*, 1994; Price *et al.*, 1994; Rokosh *et al.*, 1994). Functional experiments have shown that contractions to noradrenaline in the rat vas deferens were unaffected by chlorethylclonidine and had a relatively high affinity for WB 4101, suggesting that only the α_{1A} -adrenoceptor mediates the contraction here (Han *et al.*, 1987b; Burt *et al.*, 1992; Aboud *et al.*, 1993). Similar experiments showed that contractions to noradrenaline in the rat spleen were sensitive to chlorethylclonidine suggesting that the α_{1B} subtype mediates at least part of this contraction (Han *et al.*, 1987b; Burt *et al.*, 1992; Aboud *et al.*, 1993). The aims of this investigation were to characterize functionally the α_1 -adrenoceptor mediating contractions in the rat epididymal vas deferens and rat spleen by use of the antagonists mentioned above and to see how well their affinities correlated with those that had previously been obtained on the three cloned subtypes.

Methods

Male Sprague Dawley rats between 350–450 g were stunned and killed by cervical dislocation. The vasa deferentia were removed, associated blood vessels and mesentery were dissected away and were then bisected so that only the epididymal portion (15–20 mm in length) was used. The spleen was also removed and bisected longitudinally into two strips. All the tissues were suspended in 5 ml tissue baths containing Krebs solution of the following composition (mM): Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128, HCO_3^- 25, HPO_4^{2-} 1.2, SO_4^{2-} 1.2 and glucose 11, at 37°C and bubbled with 95% O_2 /5% CO_2 . The vas was placed under 0.5 g resting tension and equilibrated for 1 h. The spleen was placed under 1.0 g resting tension and equilibrated for 1.5 h. Changes in isometric tension were measured with Grass FT.03 transducers and recorded by Biopac Systems Inc. MP100WS for Windows.

Vas deferens

Non-cumulative additions of noradrenaline were used with a separation of 10 min between doses. The curve was then either

repeated, or repeated in the presence of cocaine and β -oestradiol (both 10^{-5} M). In other tissues, cocaine and β -oestradiol were always present and after an initial concentration-effect curve to noradrenaline had been obtained, this was either repeated in the presence of an antagonist (equilibrated for 30 min) or a concentration-effect curve to another agonist was measured. When chlorethylclonidine was the antagonist it was incubated with the tissues for 30 min and then washed out for 30 min before another concentration-effect curve was established. All the responses were calculated as a percentage of the maximum response in the initial concentration-effect curve to noradrenaline.

Spleen

An initial contraction to a concentration of noradrenaline (1×10^{-4} M) which gave a maximum response was first measured in all the tissues followed 1 h later by a cumulative concentration-effect curve to noradrenaline or phenylephrine or methoxamine. These were then repeated either as repeat control curves or in the presence of cocaine and β -oestradiol (both 10^{-5} M) or in the presence of an antagonist (equilibrated for 30 min). When chlorethylclonidine was the antagonist it was incubated with the tissues for 30 min and then washed out for 30 min. In all experiments the repeat noradrenaline and methoxamine curves were carried out 1 h after the first curve. However, the repeat phenylephrine curve was begun 2 h after the first curve because contractions to phenylephrine took much longer to return to baseline during the washout period. The responses were calculated either as a percentage of the initial maximum response to noradrenaline (1×10^{-4} M) or the maximum response in the first concentration-effect curve.

Data analysis

All the results were plotted as the mean of at least 4 separate experiments with vertical bars representing standard error of the mean (s.e.mean). For the competitive antagonists prazosin, WB 4101, 5-methyl-urapidil, phentolamine, benoxathian, spiperone, indoramin and BMY 7378, Schild plots were constructed where the x axis intercept is equal to the pA_2 (Arunlakshana & Schild, 1959). Curve fitting for the calculation of EC_{50} values by non linear regression and linear regression for the calculation of pA_2 values was performed using InPlot (GraphPAD Software, San Diego, Calif., U.S.A.). Dose-ratios were calculated using the second concentration-response curve in the absence and presence of antagonist for both the vas and spleen.

Drugs and solutions

Prazosin hydrochloride, WB 4101 (2(2,6-dimethoxyphenoxethyl)aminomethyl-1,4-benzodioxane hydrochloride), and chlorethylclonidine were donated by Pfizer Central Research, Kent; noradrenaline bitartrate, phenylephrine hydrochloride, methoxamine hydrochloride, cocaine hydrochloride, β -oestradiol and phentolamine were obtained from Sigma and 5-methyl-urapidil, benoxathian hydrochloride, spiperone hydrochloride and BMY 7378 dihydrochloride (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride) were obtained from RBI. All stock solutions were made in distilled water and diluted to working concentrations in Krebs solution except for prazosin, spiperone and β -oestradiol which were dissolved first in dimethylsulphoxide (DMSO) and then diluted in Krebs solution. Stock solutions of antagonists were stored frozen while agonists were prepared fresh each day.

Results

The potency of noradrenaline in the epididymal vas deferens and spleen was compared with that of phenylephrine and

methoxamine. On the vas deferens in the absence of uptake blocking drugs, noradrenaline and phenylephrine were equipotent and 2 times more potent than methoxamine (pD_2 5.6 ± 0.1 ; 5.6 ± 0.1 and 5.3 ± 0.1 respectively, Figure 1a). In the presence of cocaine and β -oestradiol, both at 10^{-5} M, the noradrenaline and phenylephrine concentration-effect curves were shifted to the left but the methoxamine curve remained the same. Under these conditions noradrenaline was 3 and 50 times more potent than phenylephrine and methoxamine respectively (pD_2 7.0 ± 0.1 ; 6.5 ± 0.1 and 5.3 ± 0.1 respectively, Figure 1b). All subsequent experiments with noradrenaline were carried out in the presence of the uptake inhibitors. Phenylephrine and methoxamine were both full agonists with respect to the maximum contraction evoked by noradrenaline (2.77 ± 0.18 g). In the rat spleen the same order of potency for the agonists was obtained but they were not all full agonists. Noradrenaline (pD_2 5.2 ± 0.1 , maximum tension $0.35 \text{ g} \pm 0.02$) was the most potent, followed by phenylephrine (pD_2 4.8 ± 0.1 , maximum response compared with noradrenaline of $95 \pm 2\%$) and methoxamine (pD_2 3.3 ± 0.1 but which had not reached a maximum at the highest concentration used, $73 \pm 3\%$ of noradrenaline maximum at 3×10^{-3} M) (Figure 2a). The addition of both cocaine and β -oestradiol (each 10^{-5} M) had no effect on the noradrenaline, phenylephrine or methoxamine curves (Figure 2b).

On the vas deferens, prazosin was a competitive antagonist of the noradrenaline contractions (pA_2 9.2 slope 1.02 ± 0.03 , Figure 3a). On the rat spleen prazosin at concentrations of 3×10^{-9} M and 1×10^{-8} M produced relatively small rightward

shifts in the noradrenaline curve in an apparently competitive manner but at higher concentrations (3×10^{-8} M and 1×10^{-7} M) prazosin was not a competitive antagonist, with a slope of 0.71 ± 0.08 for the Schild plot using all four concentrations (Figure 3b). Prazosin also produced a rightward shift of the phenylephrine curve and this time it acted as a competitive antagonist up to 1×10^{-7} M, (pA_2 9.2 slope 1.06 ± 0.08 , Figure 4).

Chlorethylclonidine at a concentration of 1×10^{-4} M had no effect on contractions to noradrenaline in the vas deferens (Figure 5a) but produced a 30 fold shift to the right in the noradrenaline control curve in the spleen (Figure 5b). However chlorethylclonidine (1×10^{-4} M) produced a 300 fold shift to

Figure 1 Non-cumulative concentration-effect curves for noradrenaline (●), phenylephrine (▼), and methoxamine (■), in rat epididymal vas deferens (a) without uptake blockade and (b) in the presence of cocaine (10^{-5} M) and β -oestradiol (10^{-5} M). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 2 Cumulative concentration-effect curves for noradrenaline (●), phenylephrine (▼), and methoxamine (■), in rat spleen (a) without uptake blockade and (b) in the presence of cocaine (10^{-5} M) and β -oestradiol (10^{-5} M). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 3 Antagonism of contractions to noradrenaline by prazosin in (a) rat epididymal vas deferens, control (●), + prazosin 3×10^{-9} M (▼), 1×10^{-8} M (■), 3×10^{-8} M (▲), and 1×10^{-7} M (◆) and (b) in rat spleen, control (●), + prazosin 3×10^{-9} M (▼), 1×10^{-8} M (■), 3×10^{-8} M (▲), and 1×10^{-7} M (◆). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 4 Antagonism of contractions to phenylephrine in rat spleen by prazosin: control (●), + prazosin 1×10^{-8} M (▼), 3×10^{-8} M (■), and 1×10^{-7} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

the right in the phenylephrine control curve in the spleen (Figure 5b), ten times greater than that produced against noradrenaline.

WB 4101, 5-methyl-urapidil, phentolamine, benoxathian, spiperone, indoramin and BMY 7378 were all competitive antagonists of the noradrenaline contractions in the vas deferens with pA_2 values of 9.6 (slope 1.00 ± 0.08), 8.7 (slope 1.04 ± 0.07), 8.3 (slope 1.02 ± 0.03), 9.4 (slope 1.05 ± 0.09), 7.5

Figure 5 The effect of chlorethylclonidine (30 min incubation followed by 30 min washout) on (a) contractions in the rat epididymal vas deferens to noradrenaline: control (●), + chlorethylclonidine 10^{-4} M (▼) and (b) contractions in the rat spleen to noradrenaline, control (○), + chlorethylclonidine 10^{-4} M (▼) and phenylephrine control (○), + chlorethylclonidine 10^{-4} M (▽). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 6 (a) Antagonism of contractions to noradrenaline in rat epididymal vas deferens by WB 4101. Control (●), + WB 4101 1×10^{-8} M (▼), 3×10^{-8} M (■), and 1×10^{-7} M (▲). (b) Antagonism of contractions to phenylephrine in rat spleen by WB 4101. Control (●), + WB 4101 3×10^{-8} M (▼), 1×10^{-7} M (■), and 3×10^{-7} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 7 (a) Antagonism of contractions to noradrenaline in rat epididymal vas deferens by 5-methyl urapidil. Control (●), + 5-methyl urapidil 1×10^{-8} M (▼), 3×10^{-8} M (■), and 1×10^{-7} M (▲). (b) Antagonism of contractions to phenylephrine in rat spleen by 5-methyl urapidil. Control (●), + 5-methyl urapidil 1×10^{-7} M (▼), 3×10^{-7} M (■), and 1×10^{-6} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 8 (a) Antagonism of contractions to noradrenaline in rat epididymal vas deferens by phentolamine. Control (●), + phentolamine 1×10^{-7} M (▼), 3×10^{-7} M (■), and 1×10^{-6} M (▲). (b) Antagonism of contractions to phenylephrine in rat spleen by phentolamine. Control (●), + phentolamine 3×10^{-7} M (▼), 1×10^{-6} M (■), and 3×10^{-6} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

(slope 1.02 ± 0.03), 8.4 (slope 0.97 ± 0.05) and 6.7 (slope 1.02 ± 0.04) respectively (Figures 6a–12a). They were also competitive antagonists of the phenylephrine contractions in the spleen with the following pA_2 values, WB 4101 8.1 (slope 0.95 ± 0.07), 5-methyl-urapidil 7.1 (slope 1.08 ± 0.08), phentolamine 7.3 (slope 0.98 ± 0.09), benoxathian 7.4 (slope 0.96 ± 0.07), spiperone 7.9 (slope 0.96 ± 0.05), indoramin 7.5 (slope 0.94 ± 0.08) and BMY 7378 7.4 (slope 0.93 ± 0.08) respectively (Figures 6b–12b).

Discussion

The α_1 -adrenoceptor mediating contraction of rat epididymal vas deferens and rat spleen has been characterized by use of antagonists that have been found to show some subtype specificity when used in binding experiments on membranes from cells transfected with either the cloned α_{1d} - or the α_{1b} - or the α_{1c} -adrenoceptor.

When uptake mechanisms were blocked in the epididymal vas deferens the order of potency for the agonists was noradrenaline > phenylephrine > methoxamine. The agonists had the same order of potency in contracting the rat spleen but were not affected by uptake mechanisms. The order of affinity of these agonists measured in binding studies on membranes from rat 1 fibroblast cells expressing the three cloned α_1 -adrenoceptor subtypes (Lomasney *et al.*, 1991) was the same for all three subtypes and the same as the functional order of potency in the vas deferens and spleen (Table 1). Thus this comparison is not very helpful in distinguishing between subtypes and as potency is a combination of affinity and efficacy it is not directly comparable.

In the vas deferens the competitive antagonism of noradrenaline contractions by prazosin was consistent with that expected for α_1 -adrenoceptors. The concentration-response

curve to noradrenaline was not altered by the alkylating agent chloroethylclonidine which shows that the contractions are not mediated via an α_{1B} -adrenoceptor subtype. WB 4101, 5-methyl-urapidil and benoxathian were all competitive antagonists with affinities similar to other reported values in this tissue (e.g. About *et al.*, 1993). The high affinities of WB 4101, benoxathian, 5-methyl-urapidil and phentolamine and the low affinity of spiperone are consistent with the affinities of these antagonists measured in binding studies on α_{1A} -adrenoceptors using tissues expressing either just the α_{1A} -adrenoceptor e.g. guinea-pig liver (Garcia-Sainz & Romero-Avila, 1993) or only the α_{1A} -adrenoceptor after chloroethylclonidine treatment e.g. rat hippocampus (Testa *et al.*, 1993) and rat cerebral cortex (Kenny *et al.*, 1994a). Taken together these results show that the contractions to noradrenaline in the epididymal portion of the rat vas deferens are mediated by pharmacologically defined α_{1A} -adrenoceptors. This conclusion is in line with that of a number of other workers (Han *et al.*, 1987b; Hanft & Gross, 1989; About *et al.*, 1993).

The nomenclature used so far to describe the α_1 -subtypes is that most commonly employed (see *Trends Pharmacol. Sci. Receptor Nomenclature Supplement*, 1994) although one based on different affinities for prazosin and HV723 in the vasculature has been described (Muramatsu *et al.*, 1990). The latter group has recently reported that contractions to exogenous noradrenaline in the rat epididymal vas deferens are predominantly mediated via an α_{1L} -subtype which is characterized by low affinities for prazosin, WB 4101 and HV723 (Ohmura *et al.*, 1992). However their results differ from the present results and those of other workers who have not found the lower affinity of WB 4101 prazosin and benoxathian (Table 2). In addition the α_{1A} and α_{1B} subtypes have been classified by Muramatsu as part of the α_{1H} rather than α_{1L} group and thus this conclusion (Ohmura *et al.*, 1992) is incompatible with that of the present results and that of other workers.

Figure 9 (a) Antagonism of contractions to noradrenaline in rat epididymal vas deferens by benoxathian. Control (●), + benoxathian 1×10^{-8} M (▼), 3×10^{-8} M (■), and 1×10^{-7} M (▲). (b) Antagonism of contractions to phenylephrine in rat spleen by benoxathian. Control (●), + benoxathian 3×10^{-8} M (▼), 1×10^{-7} M (■), and 3×10^{-7} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 10 (a) Antagonism of contractions to noradrenaline in rat epididymal vas deferens by spiperone. Control (●), + spiperone 1×10^{-7} M (▼), 3×10^{-7} M (■), and 1×10^{-6} M (▲). (b) Antagonism of contractions to phenylephrine in rat spleen by spiperone. Control (●), + spiperone 1×10^{-7} M (▼), 3×10^{-7} M (■), and 1×10^{-6} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 11 (a) Antagonism of contractions to noradrenaline in rat epididymal vas deferens by indoramin. Control (●), + indoramin 1×10^{-8} M (▼), 3×10^{-8} M (■), and 1×10^{-7} M (▲). (b) Antagonism of contractions to phenylephrine in rat spleen by indoramin. Control (●), + indoramin 1×10^{-7} M (▼), 3×10^{-7} M (■), and 1×10^{-6} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Figure 12 (a) Antagonism of contractions to noradrenaline in rat epididymal vas deferens by BMY 7378. Control (●), + BMY 7378 3×10^{-7} M (▼), 1×10^{-6} M (■), 3×10^{-6} M (▲), and 1×10^{-5} M (◆). (b) Antagonism of contractions to phenylephrine in rat spleen by BMY 7378. Control (●), + BMY 7378 1×10^{-6} M (▼), 3×10^{-6} M (■), and 1×10^{-5} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

Table 1 Comparison of pD₂ values for the agonists with their published pK_i on cloned subtypes

Agonist	pK _i values on cloned receptors expressed in COS-7 cells*			pD ₂ for contractile response	
	rat $\alpha_{1a/d}$	rat α_{1b}	bovine α_{1c}	vas deferens	spleen
Noradrenaline	7.0	5.0	5.0	7.0	5.2
Phenylephrine	5.8	4.6	4.3	6.5	4.8
Methoxamine	4.0	2.8	3.7	5.3	3.3

* Data from Lomasney *et al.*, 1991.

Table 2 Comparison of pA₂ values for the antagonists with their published pK_i on cloned subtypes

Antagonist	pK _i on cloned α_1 -adrenoceptors expressed in cells*			pA ₂ on contractile response	
	α_{1b}	α_{1c}	α_{1d}	vas deferens	spleen
Prazosin	9.6 ± 0.2	9.2 ± 0.2	9.4 ± 0.2	9.2	9.2
WB 4101	8.2 ± 0.1	9.5 ± 0.3	9.2 ± 0.1	9.6	8.1
5-Methyl urapidil	6.8 ± 0.3	8.8 ± 0.1	7.3 ± 0.3	8.7	7.1
Phentolamine	7.3 ± 0.2	8.1 ± 0.3	7.6 ± 0.2	8.3	7.3
Benoxathian	7.8	9.0	8.7	9.4	7.4
Sipiperone	8.3 ± 0.2	7.9 ± 0.3	7.9 ± 0.2	7.5	7.9
Indoramin	7.3 ± 0.1	8.2 ± 0.3	6.8 ± 0.2	8.4	7.5
BMY 7378	7.2	6.6	9.4	6.7	7.4

* Data are mean ± s.e.mean for values from Faure *et al.*, 1994; Forray *et al.*, 1994b; Kenny *et al.*, 1994a,b; Testa *et al.*, 1994 and Goetz *et al.*, 1995 (no s.e.mean for compounds with only one or two values). In each study the hamster α_{1b} , bovine α_{1c} and rat α_{1d} clones were used except for Forray *et al.*, (1994b) and Goetz *et al.*, (1995) where the three human α_1 -subtype clones were used.

Prazosin caused a shift in the noradrenaline curve on the spleen but was a competitive antagonist only at concentrations which produced a relatively small shift to the right. At higher concentrations of prazosin the shift was no longer in a competitive manner. Prazosin is not a subtype selective α_1 -antagonist so the response to noradrenaline in the rat spleen must contain a non α_1 -adrenoceptor component, possibly mediated by α_2 -adrenoceptors (Kenakin & Novak, 1987). To isolate only the α_1 -adrenoceptor component of the contraction, phenylephrine was therefore tried as the agonist. In this case the phenylephrine contractions, unlike those to noradrenaline, were competitively antagonized by prazosin (pA_2 9.2), up to 1×10^{-7} M. This confirmed that the phenylephrine contractions were mediated by α_1 -adrenoceptors with no non- α_1 -adrenoceptor mediated component.

The responses to noradrenaline in the spleen were antagonized by the α_{1B} alkylating agent, chlorethylclonidine (Taddei et al., 1993) at a concentration which had no effect on con-

Figure 13 Correlation of average pK_i values for the displacement of [3 H]-prazosin on cloned α_1 -adrenoceptor subtypes from Faure et al., 1994; Forray et al., 1994b; Kenny et al., 1994a,b; Testa et al., 1994 and Goetz et al., 1995 with pA_2 values for the antagonists prazosin (1), WB 4101 (2), 5-methyl urapidil (3), phentolamine (4), benoxathian (5), spiperone (6), indoramin (7) and BMY 7378 (8) against rat epididymal vas deferens noradrenaline contractions. The solid line is a linear regression fit through all the points and the dashed line has a slope equal to unity, passing through the origin.

Figure 14 Correlation of average pK_i values for the displacement of [3 H]-prazosin on cloned α_1 -adrenoceptor subtypes from Faure et al., 1994; Forray et al., 1994b; Kenny et al., 1994a,b; Testa et al., 1994 and Goetz et al., 1995 with pA_2 values for the antagonists prazosin (1), WB 4101 (2), 5-methyl urapidil (3), phentolamine (4), benoxathian (5), spiperone (6), indoramin (7) and BMY 7378 (8) against rat spleen phenylephrine contractions. The solid line is a linear regression fit through all the points and the dashed line has a slope equal to unity, passing through the origin.

Table 3 Correlation values and slopes of the correlations for the pA_2 values with their pK_i values shown in Table 2

α_1 -subtype	Rat vas deferens		Rat spleen	
	correlation (r)	slope	correlation (r)	slope
α_{1b}	0.36	0.33 ± 0.34	0.96	1.27 ± 0.14
α_{1c}	0.97	0.91 ± 0.09	0.42	0.57 ± 0.51
α_{1d}	0.09	0.09 ± 0.42	0.55	0.84 ± 0.51

tractions to noradrenaline in the rat epididymal vas deferens (see above). This suggests that at least part of the response was mediated by the α_{1B} subtype. However the response was not antagonized as much as might be expected when compared with the ability of chlorethylclonidine to reduce the B_{max} of the α_{1B} subtype in binding experiments, which is over 80% (Taddei *et al.*, 1993). Chlorethylclonidine (1×10^{-4} M) caused a much greater shift to the right of the control curve for phenylephrine than with noradrenaline. With the selective α_1 -adrenoceptor agonist the effect of chlorethylclonidine appeared to agree with that expected for the α_{1B} subtype when compared with binding data for reduction in B_{max} in tissues (Han *et al.*, 1987a; Taddei *et al.*, 1993).

The relatively low pA_2 values for WB 4101, 5-methyl urapidil, phentolamine and benoxathian and relatively high pA_2 value for spiperone that were measured against phenylephrine contractions in rat spleen (Table 2) are also consistent with the affinities of these antagonists measured in binding studies using tissues thought to express just α_{1B} -adrenoceptors e.g. rat liver (Taddei *et al.*, 1993; Kenny *et al.*, 1994), rat white fat cells (Torres-Marquez *et al.*, 1992) and rat spleen (Veenstra *et al.*, 1992). Taken together, these results show that the contractions to α_1 -adrenoceptor agonists in the rat spleen are mediated by pharmacologically defined α_{1B} -adrenoceptors, in agreement with the conclusions of other workers (Han *et al.*, 1987b; Aboud *et al.*, 1993). It is clear that at least two pharmacologically distinct α_1 -adrenoceptors exist in the rat as the spleen results are markedly different from those obtained in the rat epididymal vas deferens.

Using cell membranes from cells transfected with the cDNA for either the α_{1B} , α_{1C} or α_{1D} subtype, pK_i values for different antagonists at these α_1 -adrenoceptors have been determined in several binding studies (Faure *et al.*, 1994; Forray *et al.*, 1994b; Kenny *et al.*, 1994a,b; Testa *et al.*, 1994; Goetz *et al.*, 1995). While prazosin appears to have equal affinity for the three subtypes, other compounds show some subtype selectivity. In particular, 5-methyl urapidil and indoramin have a higher affinity for the α_{1C} subtype and BMY 7378 has a much higher affinity for the α_{1D} subtype. Table 2 compares the average affinities of antagonists from these binding studies on cell lines expressing either the cloned α_{1B} , α_{1C} or α_{1D} adrenoceptors (Faure *et al.*, 1994; Forray *et al.*, 1994b; Kenny *et al.*, 1994a,b; Testa *et al.*, 1994; Goetz *et al.*, 1995) with their pA_2 values in either the rat epididymal vas deferens or spleen obtained in this study. The pK_i values on each subtype for the antagonists were plotted against their pA_2 values on the vas deferens and spleen. Linear regression was used to see how well the pA_2 and pK_i values correlated for each cloned subtype (vas deferens Figure 13, spleen Figure 14). Correlation values (r), and slopes for each plot are shown in Table 3, a correlation value of one and slope of unity would result from the pK_i and pA_2 values for each antagonist being identical and therefore pharmacologically the same receptor.

It is clear from the correlations that the functional response in the vas deferens is unlikely to be mediated via a receptor similar to either the α_{1B} -clone or α_{1D} -clone. However there is a very good correlation between the pA_2 values and the pK_i values at the α_{1C} -clone (r 0.97, slope 0.91 ± 0.09). This suggests that the functional α_{1A} -adrenoceptor in the vas deferens and the expressed α_{1C} -clone are the same. Due to initial reports showing the bovine α_{1C} -clone to be chlorethylclonidine-sensitive and not expressed in the rat (Schwinn *et al.*, 1991) the α_{1C} -adrenoceptor clone was originally thought not to correspond to the α_{1A} -adrenoceptor found in rat tissues. However now,

following recent results, this is a possibility as the α_{1C} subtype is expressed in the rat (Perez *et al.*, 1994; Rokosh *et al.*, 1994) and this subtype may not be eliminated from a rat tissue by chlorethylclonidine treatment (Laz *et al.*, 1993; Forray *et al.*, 1994a). Further, there is evidence consistent with the presence of both α_{1C} and α_{1D} subtypes in rat lung, i.e. chlorethylclonidine treatment removed low affinity (α_{1B}) WB 1401 sites after which this antagonist could only bind to a single population of high affinity sites whereas 5-methyl urapidil could still distinguish two different affinity sites (Hiramatsu *et al.*, 1994). This can be explained by WB4101 being relatively non selective and 5-methyl urapidil having some selectivity between the α_{1D} -clone and the α_{1C} -clone. This is further supported by the RNA for all three subtypes being detected by RNase protection assay in rat lung (Rokosh *et al.*, 1994).

On the other hand the correlations showed that the subtype mediating contraction in the rat spleen was most similar to the α_{1B} -clone (r 0.96, slope 1.27 ± 0.14) and did not correlate well with the other two subtypes. This confirms that the expressed α_{1B} -clone is the same as the α_{1B} -adrenoceptor found in tissues. Although the slope of the correlation differed significantly from unity this is probably due to the narrow range of affinities for most of these antagonists on the rat spleen. This reflects the narrow range of affinities of these compounds for the expressed α_{1B} clone.

The effect of chlorethylclonidine on B_{max} has also been measured in binding studies on the cloned receptors expressed in cell lines (Schwinn *et al.*, 1990; Lomasney *et al.*, 1991; Perez *et al.*, 1991; Marshall *et al.*, 1992; Forray *et al.*, 1994a,b). It is clear that the cloned α_{1B} subtype is most sensitive to alkylation, in agreement with its effects both on B_{max} in tissues and its effects on the functional response in the spleen. However the other two α_1 clones are also partially sensitive to chlorethylclonidine and it is not yet clear whether the α_{1C} or α_{1D} clone is more so. Therefore chlorethylclonidine may be useful in identifying the α_{1B} subtype but cannot be used to distinguish between the α_{1C} and α_{1D} subtypes. This is particularly true in functional studies when a tissue with a large receptor reserve (perhaps the epididymal vas deferens) may appear to be relatively less sensitive to a receptor alkylating agent compared with a tissue with a smaller receptor reserve. This may explain why contraction of the human prostate to noradrenaline which is mediated by α_{1A} -adrenoceptors (Marshall *et al.*, 1995), appears to be more sensitive to chlorethylclonidine than the rat vas deferens.

In conclusion, contractions of the rat epididymal vas deferens to noradrenaline are mediated by a receptor similar to the classical α_{1A} -adrenoceptor as previously defined in tissues. The α_{1A} functional receptor also correlated very well pharmacologically with the expressed α_{1C} clone and appears to be the same subtype. Contractions to phenylephrine in the rat spleen are mediated by the α_{1B} -adrenoceptor. The good correlation between the affinities of the selective competitive antagonists on the expressed α_{1B} cloned receptor and the α_{1B} -adrenoceptor in the rat spleen show that these receptors are pharmacologically the same. The rat epididymal vas deferens and rat spleen therefore are useful tissues in which to study functional α_{1A} -adrenoceptors (such as those mediating contraction of human prostate, Marshall *et al.*, 1992; 1995) and α_{1B} -adrenoceptors respectively.

We thank Pfizer Central Research, Kent, for supporting this work.

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(Received November 21, 1994

Revised February 13, 1995

Accepted February 28, 1995)