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Different subtypes of α_{1A}-adrenoceptor mediating contraction of rat epididymal vas deferens, rat hepatic portal vein and human prostate distinguished by the antagonist RS 17053

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1 The α_1-adrenoceptor subtype mediating contraction of the rat hepatic portal vein to phenylephrine was characterized by use of competitive antagonists previously shown to have selectivity between the expressed α_1-subtype clones. Prazosin competitively antagonized the phenylephrine contractions with a pA_2 value of 9.2, as did WB 4101 (pA_2 9.4), 5-methyl urapidil (pA_2 8.6), indoramin (pA_2 8.4) and BMY 7378 (pA_2 6.5).

2 The pA_2 values on the rat portal vein correlated highly with their previously published pA_2 values for the α_{1A}-adrenoceptors mediating contraction of the rat epididymal vas deferens and human prostate and poorly with those for the α_{1B}- and α_{1D}-adrenoceptors mediating contraction of the rat spleen and aorta, respectively. The antagonist pA_2 values on the rat portal vein correlated highly with their previously published pK_I values for the expressed α_{1a}-clone and poorly with those for the expressed α_{1b}- and α_{1d}-clones. Therefore the results show that contraction of the rat portal vein to phenylephrine is mediated by α_{1A}-adrenoceptors.

3 The novel α_1-adrenoceptor antagonist RS 17053 had a relatively high affinity for the α_{1A}-adrenoceptors mediating contraction of the rat epididymal vas deferens (pA_2 9.5) compared with the α_{1B}-adrenoceptors in the rat spleen (pA_2 7.2) or the α_{1D}-adrenoceptors in the rat aorta (pK_B 7.1), in agreement with its selectivity for the expressed α_{1a}-clone. However, RS 17053 had over 100 fold lower affinity for the α_{1A}-adrenoceptors mediating contraction of the rat portal vein (pK_B 7.1) and human prostate (pK_B 7.1) compared with its affinity for the α_{1A}-adrenoceptors in the rat epididymal vas deferens or the expressed α_{1a}-clone.

4 The difference in affinity of RS 17053 between the rat epididymal vas deferens and rat portal vein cannot be explained by a species difference in the receptor. Therefore RS 17053 may distinguish between subtypes of the α_{1A}-adrenoceptor in the rat portal vein and human prostate compared with those in the rat epididymal vas deferens or the expressed α_{1a}-clone.

Keywords: RS 17053; α_{1A}-adrenoceptors; α_1-adrenoceptor subtypes; rat epididymal vas deferens; rat hepatic portal vein; human prostate

Introduction

Three α_1-adrenoceptor subtypes (α_{1A}-, α_{1B}- and α_{1D}-) have been characterized in rat tissues and the cDNA now thought to correspond to these subtypes (α_{1a}-, α_{1b}- and α_{1d}-, respectively) has also been cloned from both rat and human tissues (see Ford *et al.*, 1994; Minneman & Esbenshade, 1994; Heible *et al.*, 1995). Receptors defined in tissues are now denoted by upper case and cloned receptors by lower case letters (Bylund *et al.*, 1994).

Initially there was some confusion over the relationship between tissue and cloned α_1-adrenoceptor subtypes due in part to the limited number of selective antagonists available. However, this was largely resolved with a range of competitive antagonists being shown to have selectivity between the expressed α_1-subtype clones in several binding studies (e.g. Faure *et al.*, 1994; Forray *et al.*, 1994; Kenny *et al.*, 1994a, b; Testa *et al.*, 1994; Goetz *et al.*, 1995). In particular 5-methyl urapidil and indoramin are selective for the α_{1a}-subtype while BMY 7378 is selective for the α_{1d}-subtype. The affinities of these antagonists have been correlated with their affinities for α_1-adrenoceptors in various tissues in several studies. They have shown that the α_{1a}-clone corresponds with the α_{1A}-subtype mediating contraction of the rat epididymal vas deferens via activation of protein kinase C (Burt *et al.*, 1995a; 1996) and human prostate (Marshall *et al.*, 1995) while the α_{1b}-clone corresponds with the α_{1B}- subtype mediating contraction of the rat spleen via capacitative Ca^{2+} influx (Burt *et al.*, 1995a, b). At first no tissue α_1-adrenoceptors correlated with the α_{1d}-clone but it now appears that the α_1-subtype mediating contraction of the rat aorta corresponds with the α_{1d}-clone and is therefore the α_{1D}-subtype (Saussy *et al.*, 1994; Kenny *et al.*, 1995).

A novel α_1-adrenoceptor antagonist RS 17053 has recently been shown to be selective for the expressed α_{1a}-clone and α_{1A}-adrenoceptors characterized in rat tissues (Ford *et al.*, 1995; 1996). Contraction of the human prostate has also been shown to be mediated by α_{1A}-adrenoceptors (Forray *et al.*, 1994; Marshall *et al.*, 1995). However, RS 17053 had 100 fold lower affinity for the α_{1A}-adrenoceptors mediating contraction of human prostate compared with the α_{1a}-clone, suggesting that RS 17053 may distinguish between different α_1-subtypes previously characterized as α_{1A}-adrenoceptors (Ford *et al.*, 1995; 1996). α_{1A}-Adrenoceptors have also been shown to mediate contraction of the rat epididymal vas deferens (Aboud *et al.*, 1993; Burt *et al.*, 1995a) and have been suggested to mediate contraction of the rat portal vein (Lepretre *et al.*, 1994). The present study confirms that α_{1A}-adrenoceptors mediate contraction of the rat portal vein using a range of α_1-subtype selective antagonists. However, it also shows that RS 17053 may distinguish between the α_{1A}-adrenoceptors in the rat epididymal vas deferens and those in the rat portal vein and human prostate. Preliminary accounts of this work have been presented (Green *et al.*, 1996; Marshall *et al.*, 1996).

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Methods

Rat tissues

Male Sprague Dawley rats between 350–450 g were stunned and killed by cervical dislocation. A 15–20 mm length of hepatic portal vein was removed and associated connective tissue dissected away. The vasa deferentia were removed, associated blood vessels and mesentery were dissected away and were then bisected and only the epididymal portion (15–20 mm in length) was used. The spleen was removed and bisected longitudinally into two strips. The thoracic aorta was removed, connective tissue dissected away, and then cut into rings (3–5 mm long) which were denuded of endothelium. The epididymal vas deferens and spleen were set up 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 D-glucose 11. The portal vein (10–20 mm in length) was set up longitudinally in 5 ml tissue baths containing a modified high K^+ Krebs solution of the same composition except for an increase in K^+ to 50 mM and an equivalent decrease in Na^+ to 98.9 mM. This was to suppress spontaneous phasic activity. The aortic rings were set up in 10 ml tissue baths containing Krebs solution of the same composition used for the vas and spleen. All the tissues were maintained at 37°C and bubbled with 95% O_2/5% CO_2. The vas, aorta and portal vein were placed under 0.5 g resting tension and equilibrated for 1 h except for the aorta which was equilibrated for 80 min. The spleen was placed under 1.0 g resting tension and equilibrated for 1.5 h. Changes in isometric tension were measured by Grass FT.03 transducers and recorded either by Biopac Systems Inc. MP100WS for Windows or on a Grass polygraph.

For the rat epididymal vas deferens cocaine and β-oestradiol (both 10^{-5} M) were always present in the Krebs solution as they have been shown to increase the potency of noradrenaline in this tissue (Burt *et al.*, 1995a). Cocaine and β-oestradiol were not included in the Krebs solution for the rat spleen, portal vein and aorta as they were shown not to increase the potency of phenylephrine on the spleen (Burt *et al.*, 1995a) or the potency of phenylephrine or noradrenaline on the portal vein and aorta, respectively, in preliminary experiments.

For the hepatic portal vein phenylephrine was used as the agonist as part of the contraction to noradrenaline in this tissue may be mediated by α_2-adrenoceptors (Lepretre & Mironneau 1994). A concentration-response curve to cumulative additions of phenylephrine was constructed in the portal vein, the tissues were then washed for 30 min and after a further 30 min the concentration-response curve was repeated as a control or repeated in the presence of one of the following antagonists: prazosin, WB 4101, 5-methyl urapidil, indoramin and BMY 7378 (all equilibrated with the tissues for 30 min). In other tissues after a 15 min washout period RS 17053 was equilibrated with the tissues for 2 h before a second concentration-response curve to phenylephrine was measured. Preliminary experiments with RS 17053 had shown this equilibration time to be necessary in the vas deferens. A second curve to phenylephrine was also measured after 135 min without addition of RS 17053 as a control.

For the epididymal vas deferens, a concentration-response curve to non-cumulative additions of noradrenaline was constructed with a separation of 10 min between doses. The tissues were then washed for 10 min and then after a further 2 h the curve was repeated either as a control or in the presence of RS 17053 (equilibrated with the tissues for 2 h). The vas deferens was also equilibrated with RS 17053 (1×10^{-8} M and 3×10^{-7} M) for 2 h and then tissues were washed for 30 min before a second concentration-response curve to noradrenaline was constructed, with no further additions of RS 17053 before subsequent doses.

For the spleen, an initial contraction to noradrenaline (10^{-4} M, which gave a maximum response) was first measured followed one hour later by a cumulative concentration-response curve to phenylephrine. The tissues were then washed for 1 h and then after a further 2 h the phenylephrine curve was either repeated as a control curve or in the presence of RS 17053 (equilibrated with the tissues for 2 h).

For the thoracic aorta, a response to noradrenaline (10^{-7} M), which produced a submaximal contraction was measured and assessed for stability over 15 min. If the response was stable, acetylcholine (10^{-6} M) was added. When the contraction was unstable or acetylcholine produced a relaxation >10% then the tissue was discarded. After 20 min another contraction to noradrenaline (10^{-7} M) was measured and this was followed 30 min later by a cumulative concentration-effect curve to noradrenaline. Tissues were then washed for 45 min and then after a further 2 h the noradrenaline curve was either repeated as a control curve or in the presence of RS 17053 (equilibrated with the tissues for 2 h). In some tissues after the initial concentration-response curve to noradrenaline the aorta was equilibrated with RS 17053 (1×10^{-5} M) for 45 min before a second concentration-response curve to noradrenaline was measured. The tissues were then washed for 90 min before a third concentration-response curve to noradrenaline was constructed.

Human prostate

Prostatic chips taken from patients undergoing transurethral resection for benign prostatic hyperplasia (age 60–85, $n=5$) were collected in Tyrode solution and stored overnight at 4°C for experimental use the next day. Prostatic chips (about 20 mm × 4 mm × 2 mm) were selected which contained the most smooth muscle. They were suspended in Tyrode solution (composition mM: Na^+149, Cl^-141, HCO_3^-12, D-glucose 5.6, HPO_4^{2-}0.3, K^+2.7, Mg^{2+}0.5 and Ca^{2+}1.8) at 37°C in 5 ml tissue baths and bubbled with 95% O_2/5% CO_2. Cocaine and β-oestradiol were not included in the Tyrode solution as they were shown not to increase the potency of noradrenaline in the human prostate (Marshall *et al.*, 1995). The strips were placed under 1 g resting tension, and equilibrated for 1 h. Changes in isometric tension were measured by Grass FT.03 transducers and recorded by Biopac Systems Inc. MP100WS for Windows.

Contractions to cumulative additions of noradrenaline were measured in all tissues. The tissues were then washed for 30 min and then after a further 2 h a curve was either repeated, or repeated in the presence of RS 17053 (equilibrated with the tissues for 2 h).

Drugs and solutions

RS 17053 (*N*-[2-(2cyclopropylmethoxyphenoxy)ethyl]5-chloro-α, α-dimethyl-1 *H*-indole3-ethylamine hydrochloride) was donated by Roche Bioscience. Prazosin hydrochloride and WB 4101 (2(2,6-dimethoxyphenoxyethyl)amino-methyl-1,4-benzodioxane hydrochloride) were donated by Pfizer Central Research, Kent. Noradrenaline bitartrate, phenylephrine hydrochloride, cocaine hydrochloride and β-oestradiol were obtained from Sigma and 5-methyl-urapidil and BMY 7378 (8[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5] decane-7,9-dione dihydrochloride) dihydrochloride were obtained from RBI. All stock solutions were made in distilled water and diluted to working concentrations in Krebs solution for use with rat tissues or Tyrode solution for the human prostate. However, prazosin, RS 17053 and β-oestradiol were dissolved first in dimethyl sulphoxide (DMSO) and then diluted in Krebs or Tyrode solution. Stock solutions of antagonists were stored frozen while agonists were prepared fresh each day.

Data analysis

Responses in all tissues were calculated as a percentage of the maximum response in the initial concentration-response curve. They were plotted as the mean of at least 4 separate experi-

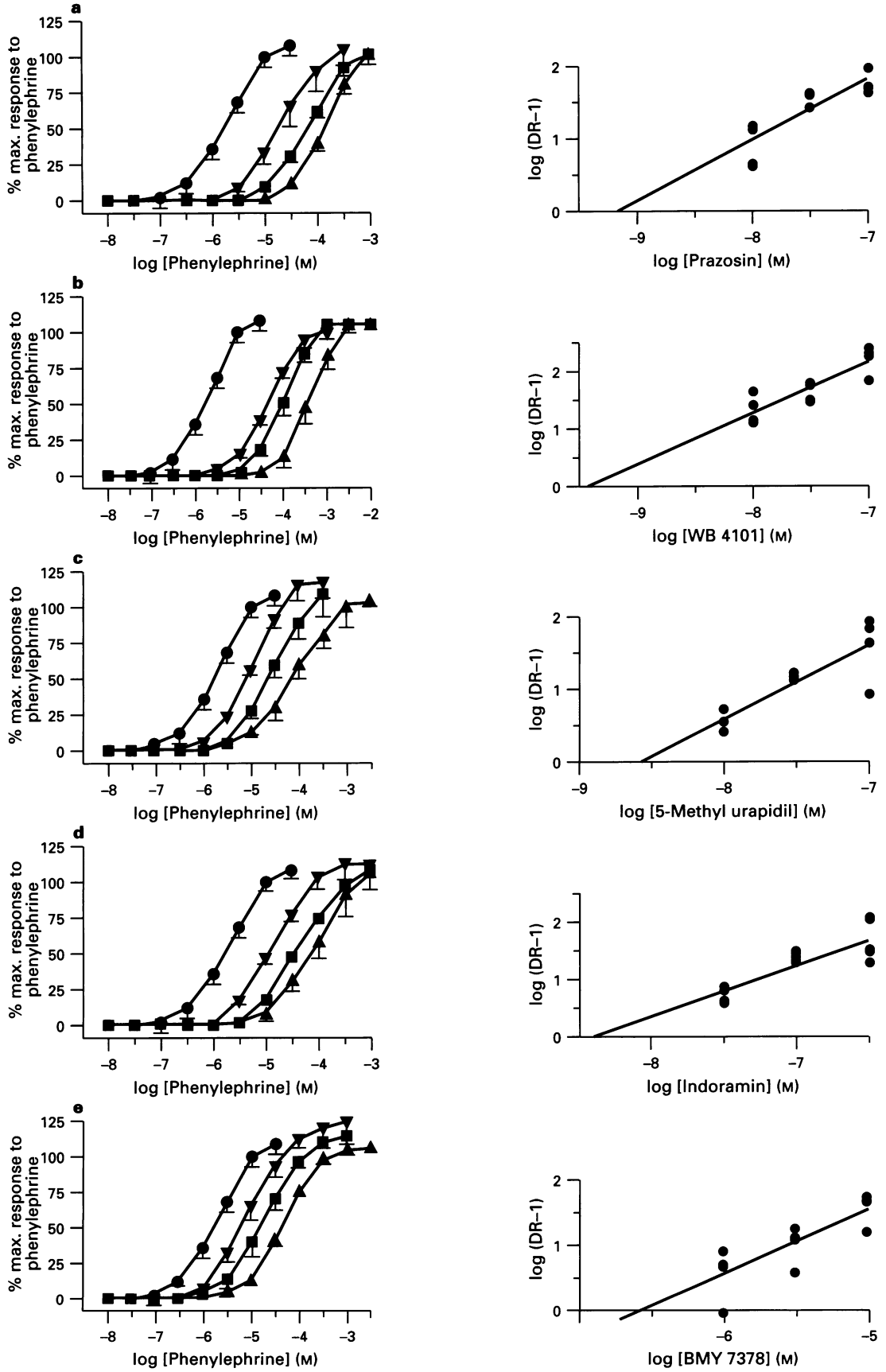

Figure 1 Antagonism of contractions to phenylephrine in rat portal vein by (a) prazosin, control (●), + prazosin 1×10^{-8} M (▼), 3×10^{-8} M (■), 1×10^{-7} M (▲). (b) WB 4101, control (●), + WB 4101 1×10^{-8} M (▼), 3×10^{-8} M (■), 1×10^{-7} M (▲). (c) 5-Methyl urapidil, control (●), + 5-methyl urapidil 1×10^{-8} M (▼), 3×10^{-8} M (■), 1×10^{-7} M (▲). (d) Indoramin, control (●), + indoramin 3×10^{-8} M (▼), 1×10^{-7} M (■), 3×10^{-7} M (▲). (e) BMY 7378, control (●), + BMY 7378 1×10^{-6} M (▼), 3×10^{-6} M (■), 1×10^{-5} M (▲). Each plot represents the mean with s.e. mean of at least 4 separate experiments. Schild plots for each antagonist constructed using concentration-ratios from (a), (b), (c), (d) and (e).

ments with vertical bars representing standard error of the mean (s.e.mean). For the competitive antagonists prazosin, WB 4101, 5-methyl urapidil, indoramin and BMY 7378, Schild plots were constructed where the x axis intercept is equal to the pA_2 (Arunlakshana & Schild, 1959). pK_B values were equal to log (concentration-ratio -1) $-$ log [antagonist]. 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 by use of InPlot (GraphPAD Software, San Diego, Ca, U.S.A.). Concentration-ratios were calculated from the second concentration-response curve in the absence and presence of antagonist for the rat vas, spleen and human prostate while for the rat portal vein and aorta they were calculated from the first and second curves within the same tissue. Where agonist potencies have been given as pEC_{50} values this is equal to $-$log of the EC_{50} value for the agonist.

Results

Rat hepatic portal vein

The α_1-adrenoceptor subtype mediating contraction of the rat portal vein was characterized by use of antagonists that have been shown to have selectivity between the cloned α_1-subtypes (Faure *et al.*, 1994; Forray *et al.*, 1994; Kenny *et al.*, 1994a, b; Testa *et al.*, 1994; Goetz *et al.*, 1995).

The modified high K^+ Krebs solution abolished nearly all spontaneous phasic activity observed on the portal vein when the tissues were set up in normal Krebs solution (results not shown). All further experiments were carried out in the modified Krebs solution. Cumulative additions of phenylephrine (10^{-8} M -10^{-4} M) produced a tonic contraction of the portal vein (pEC_{50} 5.7 ± 0.1, maximum response 0.22 ± 0.01 g, mean $\pm$ s.e.mean) and the repeat concentration-response curve after 1 h was not significantly different.

The selective α_1-adrenoceptor antagonist prazosin produced concentration-dependent rightward shifts in the phenylephrine curve and the Schild plot gave a pA_2 value of 9.2 (slope 0.84 ± 0.16) (Figure 1a). The phenylephrine contractions were also competitively antagonized by WB 4101 ($pA_2$9.4, slope 0.89 ± 0.16, Figure 1b), 5-methyl urapidil ($pA_2$8.6, slope 1.02 ± 0.19, Figure 1c), indoramin ($pA_2$8.4, slope 0.87 ± 0.15, Figure 1d) and BMY 7378 ($pA_2$6.5, slope 1.02 ± 0.23, Figure 1e).

The antagonist pA_2 values given above for the portal vein were correlated with those obtained using the same compounds against α_1-adrenoceptor mediated contractions of the rat epididymal vas deferens (Burt *et al.*, 1995a), human prostate (Marshall *et al.*, 1995), rat spleen (Burt *et al.*, 1995a) and rat aorta (Kenny *et al.*, 1995) (Figure 2). Correlation coefficients (r) and slopes of the correlation plots in Figure 2 are

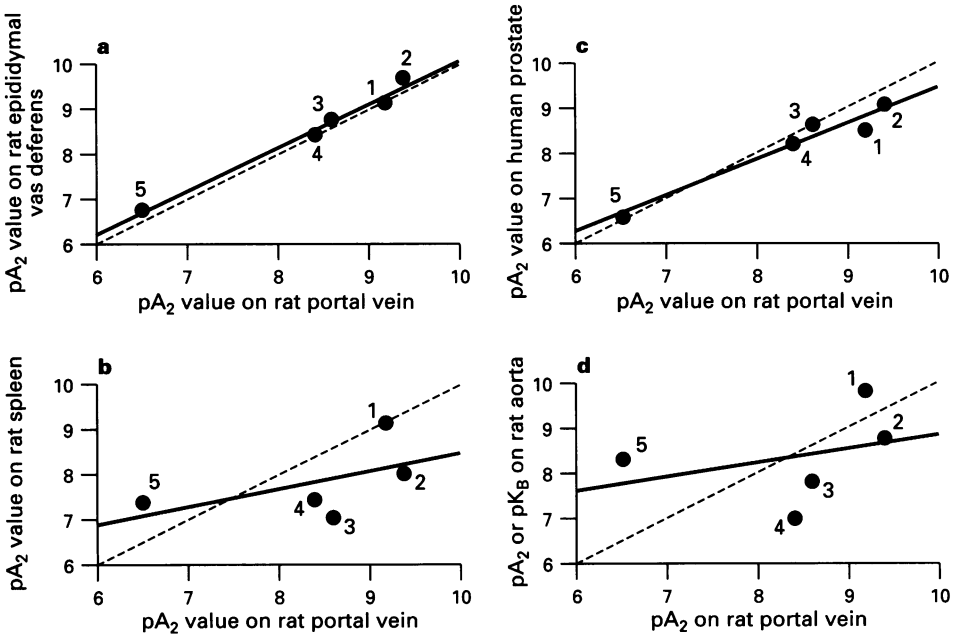

Figure 2 Correlation of pA_2 values for the antagonists prazosin (1), WB 4101 (2), 5-methyl urapidil (3), indoramin (4) and BMY 7378 (5) on (a) rat epididymal vas deferens (Burt *et al.*, 1995a), (b) rat spleen (Burt *et al.*, 1995a), (d) rat aorta (Kenny *et al.*, 1995) and (c) human prostate (Marshall *et al.*, 1995) with their pA_2 values on contractions to phenylephrine in rat portal vein. 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 1 Comparison of pA_2 values for α_1-adrenoceptor antagonists on the rat portal vein with their published pK_i on cloned subtypes

Antagonist	*pK_i on cloned α_1-adrenoceptors expressed in cells**			*pA_2 rat portal vein*
	α_{1a}	α_{1b}	α_{1d}	
Prazosin	9.2 ± 0.2	9.6 ± 0.2	9.4 ± 0.2	9.2
WB4101	9.5 ± 0.3	8.2 ± 0.1	9.2 ± 0.1	9.4
5-Methyl urapidil	8.8 ± 0.1	6.8 ± 0.3	7.3 ± 0.3	8.6
Indoramin	8.2 ± 0.3	7.3 ± 0.1	6.8 ± 0.2	8.4
BMY 7378	6.6	7.2	9.4	6.5

*Data are mean $\pm$ s.e.mean for values from Faure *et al.* (1994); Forray *et al.* (1994); 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 bovine α_{1a} hamster α_{1b} and rat α_{1d} clones were used except for those by Forray *et al.* (1994b) and Goetz *et al.* (1995) where the three human α_1-subtype clones were used.

given in Table 2. The pA_2 values were also correlated with the average pK_i values for these antagonists on the expressed cloned α_1-adrenoceptor subtypes from Faure *et al.* (1994), Forray *et al.* (1994), Kenny *et al.* (1994a, b), Testa *et al.* (1994) and Goetz *et al.* (1995) shown in Table 1 (Figure 3). Correlation coefficients (r) and slopes of the correlation plots in Figure 3 are given in Table 2. This analysis suggests that the α_1-adrenoceptor mediating contraction of the rat portal vein is the α_{1A}-subtype. The antagonist pA_2 values for the α_{1A}-adrenoceptor mediated contractions of the rat epididymal vas deferens (Burt *et al.*, 1995a) and human prostate (Marshall *et al.*, 1995) also correlated well ($r=0.96$, slope 1.18 ± 0.15).

Table 2 Correlation coefficients and slopes of the correlation plots for antagonist pA_2 values on the rat portal vein with either their pA_2 values on other tissues (from Figure 2) or with their pK_i values on the expressed α_1-clones (from Figure 3)

pA_2 values on the rat portal vein against	*Correlation* (r)	*Slope*
Rat epididymal vas deferens	1.00	0.97 ± 0.05
Human prostate	0.97	0.79 ± 0.10
Rat spleen	0.53	0.38 ± 0.35
Rat aorta	0.33	0.30 ± 0.50
Expressed α_{1a}-clone	0.99	0.99 ± 0.08
Expressed α_{1b}-clone	0.54	0.53 ± 0.47
Expressed α_{1d}-clone	−0.09	-0.10 ± 0.63

RS 17053

Preliminary experiments indicated that RS 17053 required a 2 h equilibration period in the rat epididymal vas deferens. RS 17053 was therefore equilibrated for 2 h with all tissues. In the rat epididymal vas deferens RS 17053 produced apparently competitive shifts in the concentration-response curves to noradrenaline up to 1×10^{-8} M but at higher concentrations a reduction in maximum response was observed (Figure 4a). When dose-ratios from the 3 lowest concentrations of RS 17053 on the rat epididymal vas deferens were used a pA_2 value of 9.5 (slope of Schild plot 1.07 ± 0.08) was obtained (Figure 4b). The vas deferens was also equilibrated with RS 17053 (1×10^{-8} M and 3×10^{-7} M) for 2 h and then washed for 30 min with no further additions of RS 17053 before subsequent noradrenaline doses. In these tissues the concentration-response curves to noradrenaline were shifted to the right compared with the control and the maximum response was also decreased by RS 17053 3×10^{-7} M (Figure 4c).

In the rat spleen and aorta RS 17053 produced dose-dependent shifts in the phenylephrine and noradrenaline concentration-response curves, respectively, but only at higher concentrations compared with the epididymal vas deferens (Figures 5a and 6a, respectively). There was no apparent reduction in maximum response in these tissues at the highest concentrations of RS 17053 used. In the rat spleen and aorta RS 17053 had a pA_2 value of 7.2 (slope 1.04 ± 0.04) and 6.6 (slope 1.96 ± 0.36) respectively (Figures 5b and 6b, respectively). The lowest concentration of RS 17053 used on the rat aorta (1×10^{-6} M) produced a pK_B value of 7.1 ± 0.1. When RS 17053 (10^{-5} M) was equilibrated with the rat aorta for 45 min

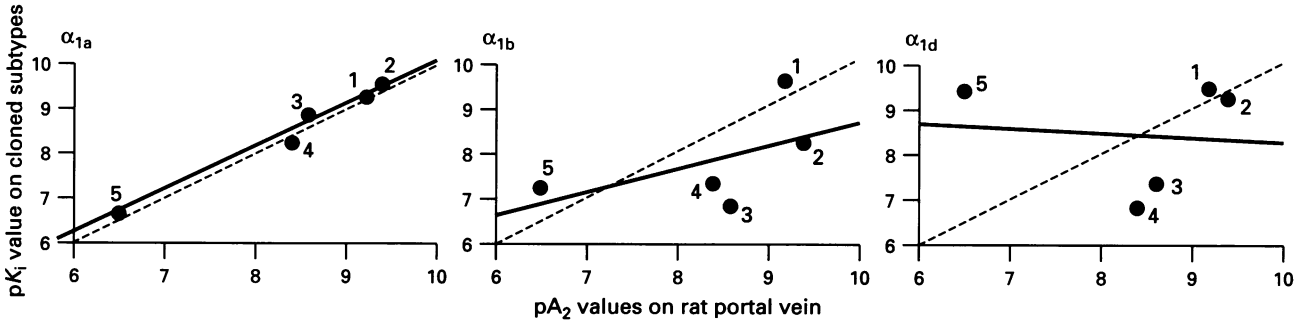

Figure 3 Correlation of average pK_i values for the displacement of [^{3}H]-prazosin on cloned α_1-adrenoceptor subtypes (from Faure *et al.*, 1994; Forray *et al.*, 1994; Kenny *et al.*, 1994a, b; Testa *et al.*, 1994 and Goetz *et al.*, 1995) with pA_2 values against contractions to phenylephrine on rat portal vein for the antagonists prazosin (1), WB 4101 (2), 5-methyl urapidil (3), indoramin (4) and BMY 7378 (5). 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.

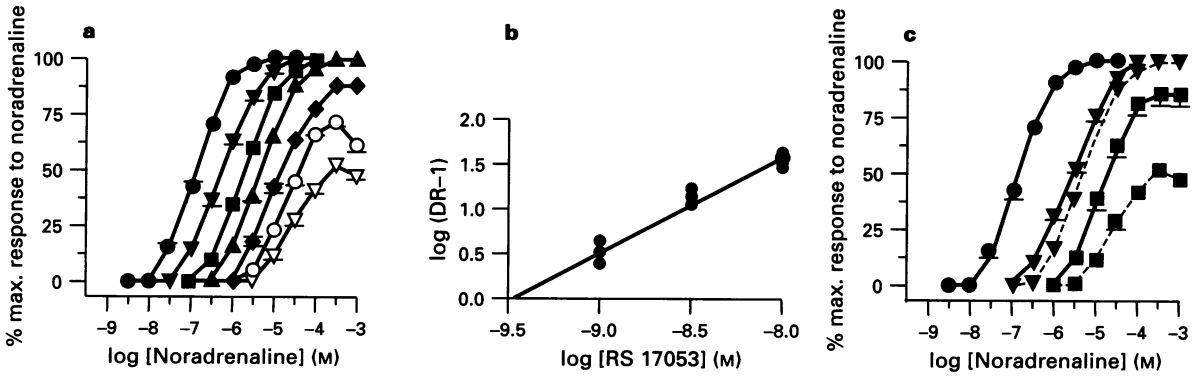

Figure 4 (a) Antagonism by RS 17053 of contractions to noradrenaline in rat epididymal vas deferens. Control (●), + RS 17053 1×10^{-9} M (▼), 3×10^{-9} M (■), 1×10^{-8} M (▲), 3×10^{-8} M (◆), 1×10^{-7} M (○) and 3×10^{-7} M (▽). Each plot represents the mean with s.e.mean of at least 4 separate experiments. (b) Schild plot for RS 17053 using concentration-ratios from the 3 lowest concentrations of RS 17053 in (a). (c) The effect of 30 min washout after a 2 h equilibration with RS 17053 (and with no further re-additions of RS 17053 before subsequent noradrenaline doses) on contractions to noradrenaline in the rat epididymal vas deferens. Control without addition of RS 17053 (●), and solid line plots with washout or dotted line plots without washout of RS 17053, 1×10^{-8} M (▼), 3×10^{-7} M (■). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

and then washed out for 90 min the concentration-response curve after this washout was shifted to the right compared with the control curve (Figure 6c).

In the rat portal vein RS 17053 produced rightward shifts in the phenylephrine concentration-response curve but only at higher concentrations compared with the epididymal vas deferens (Figure 7). However, RS 17053 did not antagonize the phenylephrine contractions competitively in this tissue, producing only a 3 fold shift at 10^{-7} M but around 100 fold at 10^{-6} M and a reduction of the maximum response even at the lowest concentration used. The lowest concentration of RS 17053 used (1×10^{-7} M) produced a pK_B value of 7.1 ± 0.1 in the rat portal vein.

In the human prostate RS 17053 antagonized the noradrenaline contractions but again only at higher concentrations compared with the rat epididymal vas deferens and reduced the maximum responses to noradrenaline at all the concentrations used (Figure 8). The lowest concentration of RS 17053 used (1×10^{-7} M) produced a pK_B value of 7.1 ± 0.1 in the human prostate.

Discussion

The first part of this investigation characterized the α_1-adrenoceptor subtype mediating contraction of the rat hepatic portal vein to phenylephrine using a range of competitive antagonists shown to have selectivity between the expressed α_1-adrenoceptor subtype clones in binding studies (Faure *et al.*, 1994; Forray *et al.*, 1994; Kenny *et al.*, 1994a, b; Testa *et al.*, 1994; Goetz *et al.*, 1995). The affinities of these antagonists for the α_1-subtype mediating contractions of the rat epididymal vas deferens and the human prostate have previously been shown to correlate well with their average affinities for the expressed α_{1a}-clone (Faure *et al.*, 1994; Forray *et al.*, 1994; Kenny *et al.*, 1994a, b; Testa *et al.*, 1994; Goetz *et al.*, 1995) suggesting that both these responses are mediated by the α_{1A}-subtype (Burt *et al.*, 1995a; Marshall *et al.*, 1995). The affinities of the antagonists for the α_1-adrenoceptors mediating con-

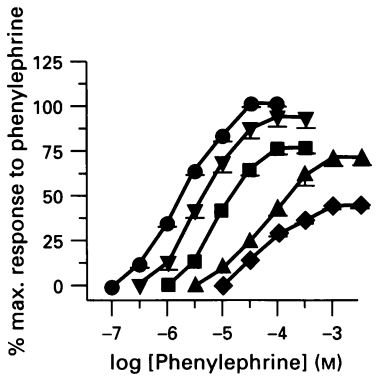

Figure 7 Antagonism by RS 17053 of contractions to phenylephrine in rat portal vein. Control (●), + RS 17053 1×10^{-7} M (▼), 3×10^{-7} M (■), 1×10^{-6} M (▲) and 3×10^{-6} M (◆). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

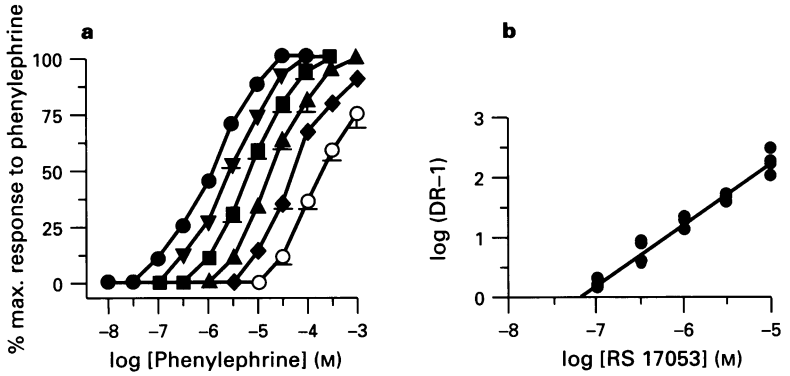

Figure 5 (a) Antagonism by RS 17053 of contractions to phenylephrine in rat spleen. Control (●), + RS 17053 1×10^{-7} M (▼), 3×10^{-7} M (■), 1×10^{-6} M (▲), 3×10^{-6} M (◆) and 1×10^{-5} M (○). Each plot represents the mean with s.e.mean of at least 4 separate experiments. (b) Schild plot for RS 17053 using concentration-ratios from (a).

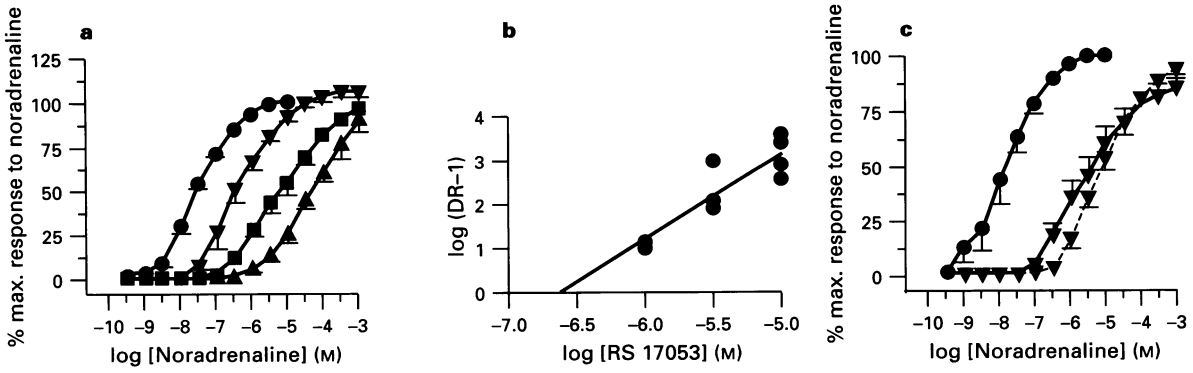

Figure 6 (a) Antagonism by RS 17053 of contractions to noradrenaline in rat aorta. Control (●), + RS 17053 1×10^{-6} M (▼), 3×10^{-6} M (■) and 1×10^{-5} M (▲). Each plot represents the mean with s.e.mean of at least 4 separate experiments. (b) Schild plot for RS 17053 using concentration-ratios from (a). (c) The effect of 90 min washout after 45 min equilibration with RS 17053 on contractions to noradrenaline in the rat aorta. Control without addition of RS 17053 (●), and solid line plot with washout or dotted line plot without washout of RS 17053, 1×10^{-5} M (▼). Each plot represents the mean with s.e.mean of at least 4 separate experiments.

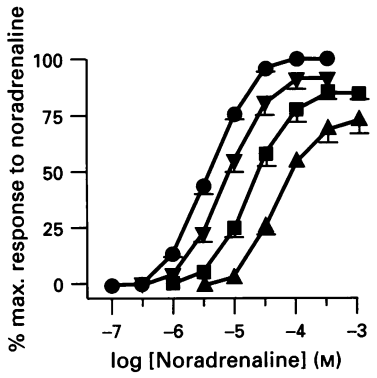

Figure 8 Antagonism by RS 17053 of contractions to noradrenaline in human prostate. Control (●), + RS 17053 1×10^{-7} M (▼) 3×10^{-7} M (■) and 1×10^{-6} M (▲). Each plot represents the mean with s.e.mean of at least 3 separate experiments.

traction of the rat spleen however correlated best with their affinities for the expressed α_{1b}-clone and is therefore the α_{1B}-subtype (Burt *et al.*, 1995a).

Prazosin competitively antagonized the phenylephrine contractions in the portal vein with a pA_2 value of 9.2, consistent for α_1-adrenoceptors and similar to the value of 8.9 obtained by Schwietert *et al.* (1991). WB 4101, 5-methyl urapidil, indoramin and BMY 7378 were also competitive antagonists in this tissue. WB 4101, 5-methyl urapidil and indoramin all had relatively high affinities, consistent with those found for α_{1A}-adrenoceptors in other tissues (Burt *et al.*, 1995a; Marshall *et al.*, 1995), while the α_{1D}-subtype selective antagonist BMY 7378 had a relatively low affinity compared with that expected for the α_{1D}-subtype (Goetz *et al.*, 1995; Kenny *et al.*, 1995). The affinities of these antagonists correlated highly with their affinities for the α_{1A}-adrenoceptors mediating contraction of the rat epididymal vas deferens and human prostate (Burt *et al.*, 1995a; Marshall *et al.*, 1995) and poorly with the α_{1B}- and α_{1D}-mediated contractions of the rat spleen and aorta, respectively (Burt *et al.*, 1995a; Kenny *et al.*, 1995). They also correlated well with their average published affinities for the cloned α_{1a}-adrenoceptor and poorly with those for the cloned α_{1b}- and α_{1d}-adrenoceptor. Therefore these results suggest that the α_1-subtype mediating contraction of the rat portal vein is the α_{1A}-adrenoceptor, in agreement with the conclusion of Lepretre *et al.* (1994).

The novel α_1-adrenoceptor antagonist RS 17053 was shown in binding studies to be selective for the expressed α_{1a}-subtype clone (Ford *et al.*, 1995). However, RS 17053 was shown to have a 100 fold lower than expected affinity for the α_1-adrenoceptors mediating contraction of the human prostate (Ford *et al.*, 1995) which have previously been characterized as the α_{1A}-subtype (Forray *et al.*, 1994; Marshall *et al.*, 1995). Therefore the second part of this investigation measured the affinity of RS 17053 for the α_{1A}-adrenoceptors mediating contraction of the rat epididymal vas deferens (Burt *et al.*, 1995a), rat portal vein and human prostate (Marshall *et al.*, 1995) as well as the α_{1B}-adrenoceptors mediating contraction of the rat spleen (Burt *et al.*, 1995a) and the α_{1D}-adrenoceptors which predominantly mediate contraction of the rat aorta (Kenny *et al.*, 1995).

RS 17053 had a high affinity for the α_{1A}-adrenoceptors mediating contraction of the rat epididymal vas deferens similar to that for the α_{1a}-subtype clone and a lower affinity for the α_1-adrenoceptors mediating contraction of the rat spleen and aorta. This suggested that it was functionally selective for the α_{1A}-subtype in agreement with its relative affinity for the cloned α_1-subtypes (Ford *et al.*, 1995). However, RS 17053 caused a reduction in maximum response at the higher concentrations used in the epididymal vas deferens suggesting that it may not be a competitive antagonist. The slope of the Schild plot for RS 17053 in the rat aorta was significantly steeper than unity, suggesting non-competitive antagonism.

In the rat portal vein and human prostate the affinity of RS 17053 for the α_{1A}-adrenoceptors mediating their contraction was about 100 fold lower compared with its affinity for the α_{1A}-adrenoceptors in the rat epididymal vas deferens or the expressed α_{1a}-subtype clone. RS 17053 also caused a reduction in maximum response in all three tissues. The 100 fold lower affinity of RS 17053 in the rat portal vein compared with the rat epididymal vas deferens cannot be explained by a species difference in the α_{1A}-subtype. The low affinity of RS 17053 in the human prostate cannot be the result of a species difference in the human α_{1a}-clone either as RS 17053 was shown to have a high affinity for both human and bovine α_{1a}-clones (Ford *et al.*, 1996). Therefore RS 17053 may identify different α_{1A}-subtypes in the rat portal vein and human prostate compared with the α_{1A}-subtype in the rat epididymal vas deferens and the α_{1a}-subtype clone.

Characterization of α_1-adrenoceptor subtypes is usually in accordance with the α_{1A}-, α_{1B}-, α_{1D}- subtype classification, which has been substantiated by the cloning of their corresponding cDNA (see Heible *et al.*, 1995). One feature of this classification is that prazosin does not distinguish between these subtypes. However, another system of α_1-adrenoceptor subtype classification exists, based on high and low affinities for prazosin, α_{1H}- and α_{1L}-, respectively (Muramatsu *et al.*, 1990). It has been proposed by this group that the α_1-adrenoceptor mediating contraction of the human prostate is the α_{1L}-subtype based on a relatively low affinity for prazosin in functional experiments (Muramatsu *et al.*, 1994). The same group also found a low prazosin affinity for the α_1-adrenoceptor mediating contraction of the rat vas deferens ($pA_2$8.3 for the epididymal portion and 8.4 for the prostatic portion, Ohmura *et al.*, 1992) and again suggested this indicated the α_{1L}-subtype. This is in contrast to pA_2 values for prazosin obtained in other studies for either the whole vas deferens ($pA_2$9.3, Aboud *et al.*, 1993; $pA_2$9.3, Teng *et al.*, 1994) or for the epididymal vas deferens ($pA_2$9.2, Burt *et al.*, 1995a). So these results cannot be explained by different parts of the vas having been used. Further conflict arises from the fact that Ohmura *et al.* (1992) and Muramatsu *et al.* (1994) both postulated that the α_{1A}-adrenoceptor is a form of the α_{1H}-subtype. Therefore their identification of a functional α_{1L}-subtype in the human prostate and rat vas deferens cannot be reconciled with the characterization of α_{1A}-adrenoceptors mediating contraction of the human prostate (Forray *et al.*, 1994; Marshall *et al.*, 1995) and rat vas deferens (Aboud *et al.*, 1993; Burt *et al.*, 1995).

Ford *et al.* (1996) have also suggested that the α_1-adrenoceptor mediating contraction of the human prostate is the α_{1L}-subtype, with RS 17053 as well as prazosin having lower affinity for the α_{1L}-subtype compared with the α_{1A}-subtype. However, in functional studies where a 'low' prazosin affinity has been obtained in the human prostate e.g. $pA_2$8.5 (Marshall *et al.*, 1992), and $pA_2$8.7 (Ford *et al.*, 1996) the values are not as low as that predicted in binding studies for the α_{1L}-subtype (pK_i8.0, Ohmura & Muramatsu, 1995), only being about three fold lower than values obtained in functional studies of other α_{1A}-adrenoceptors (Aboud *et al.*, 1993; Burt *et al.*, 1995a). In other studies pA_2 values for prazosin of 9.0 or greater in human prostate have been obtained (Chapple *et al.*, 1989; Forray *et al.*, 1994; Hatano *et al.*, 1994; Teng *et al.*, 1994).

One of the main problems in determining whether prazosin does distinguish between α_1-adrenoceptor subtypes is that the pA_2 values published cover a continuous range of values rather than falling into discrete groups. For example, pA_2 values of 8.3, 8.1 and 8.1 have been documented for α_1-adrenoceptors in the rabbit trigone, urethra and prostate, respectively (Honda *et al.*, 1985), 8.5 in rabbit prostate (Williams & Clarke, 1993), 8.9 in rat anococcygeus (Ford *et al.*, 1993) and rat portal vein (Schwietert *et al.*, 1991), 9.2 in rat epididymal vas deferens, spleen and portal vein (Burt *et al.*, 1995a; this study), 9.3, 9.5, and 9.6 in rat perfused kidney, perfused mesentery and aortic

rings respectively (Ford *et al.*, 1996) and 9.8 in rat aorta (Kenny *et al.*, 1995). Both Schwietert *et al.* (1991) and Ford *et al.* (1996) have suggested that a pA_2 of 8.9 for prazosin represents a 'low' value, indicating an α_{1L}-adrenoceptor subtype in the rat portal vein and anococcygeus, respectively. However, 8.9 is in the middle of a spread of pA_2 values for prazosin and so to characterize the α_{1L}-subtype according to whether or not a pA_2 value of less than 9.0 is obtained (Muramatsu *et al.*, 1990) is clearly impractical. This is further shown by the pA_2 value of 9.2 obtained for prazosin on the rat portal vein in this study being consistent with the α_{1A}-subtype and yet representing less than a 2 fold higher affinity compared with the value of 8.9 found for prazosin in this tissue by Schwietert *et al.* (1991).

One explanation for the range of reported affinities for prazosin could be differing proportions of both α_{1L}- and α_{1H}-adrenoceptors in tissues. This could result in the wide range of prazosin affinities but might be associated with non-competitive antagonism which has not been described. Another possible explanation for the range of reported prazosin affinities might be that human (but not rat) α_1-acid glycoprotein can inhibit prazosin (and WB 4101) binding to α_1-adrenoceptors and therefore reduce their affinity for these receptors. The presence of this glycoprotein in functional studies might therefore result in lower apparent pA_2 values for these antagonists in human tissues (Chiang *et al.*, 1991; Qin & Øie, 1994).

RS 17053 has a low affinity for the α_{1A}-adrenoceptors in the rat portal vein and human prostate, which suggests that they may both be the same subtype, being similar to, but not identical with the expressed α_{1a}-clone. However if the 'low' prazosin affinity obtained in some studies for the α_{1A}-adrenoceptors mediating contraction of human prostate (e.g. Marshall *et al.*, 1992) also indicates that the α_{1A}-subtype in the prostate is different from the expressed α_{1a}-clone, then this would suggest that the α_{1A}-adrenoceptors in the rat portal vein and human prostate are not the same either, as a pA_2 value of 9.2 was found for prazosin in the portal vein. On this logic the results with RS 17053 and prazosin suggest that there are three different α_1-adrenoceptors which have been characterized as the α_{1A}-subtype (including those in the rat epididymal vas deferens) using other subtype selective antagonists.

As the α_1-adrenoceptors mediating contraction of the rat epididymal vas deferens, portal vein and human prostate all correlated well with the α_{1a}-subtype clone and with each other when the affinities of α_1-subtype selective antagonists of WB 4101, 5-methyl urapidil, indoramin and BMY 7378 were compared, they have all been characterized as α_{1A}-adrenoceptors. This degree of pharmacological similarity between the α_{1a}-clone and the subtypes in the rat epididymal vas deferens, portal vein and human prostate therefore suggests that RS 17053 may discriminate between subtypes of the α_{1A}-adrenoceptor rather than revealing an α_1-adrenoceptor distinct from the α_{1A}- α_{1B}- and α_{1D}-subtypes. This would also fit better with the molecular biology of α_1-adrenoceptors as the cDNA for three α_1-subtypes has been cloned. Therefore it is possible that while the α_{1A}-subtype in the rat epididymal vas deferens is the same as the expressed rat α_{1a}-clone the α_1-subtypes in the rat portal vein and human prostate may be splice variants of the α_{1a}-clone, three of which have been identified in human prostate (and all have equal affinity for prazosin in binding studies when expressed in CHO cells) (Hirasawa *et al.*, 1995).

RS 17053 produced a reduction in maximum response in the rat epididymal vas deferens, portal vein and human prostate. The reduction in maximum response occurred only at the higher concentrations of RS 17053 ($>10^{-8}$ M) in the epididymal vas deferens, which produced greater than 100 fold rightward shifts in the concentration-response curves but in the rat portal vein and human prostate the reduction occurred with relatively small rightward shifts in the concentration effect curves. RS 17053 may only reduce the maximum response in the epididymal vas deferens at the higher concentrations used in this tissue because the rat epididymal vas deferens has a relatively large α_1-adrenoceptor receptor reserve (Diaz-Toledo & Marti, 1988) compared with the other two tissues. When the rat epididymal vas deferens and aorta were washed after equilibration with RS 17053, the following concentration-response curves were still shifted to the right. This suggests that RS 17053 may have a very slow rate of dissociation from the receptor.

In conclusion, the α_1-adrenoceptors mediating contraction of the rat portal vein (this study) rat epididymal vas deferens (Burt *et al.*, 1995a) and human prostate (Marshall *et al.*, 1995) have all been shown to correlate highly with the expressed α_{1a}-clone when the affinities of a range of α_1-subtype selective antagonists were compared. However, RS 17053 was 100 fold lower in affinity for the α_{1A}-adrenoceptors in the rat portal vein and human prostate compared with either the expressed α_{1a}-clone or the α_{1A}-adrenoceptors in the rat epididymal vas deferens. The difference in affinity of RS 17053 between the rat epididymal vas deferens and rat portal vein cannot be explained away as due to a species difference in receptor. Therefore RS 17053 may distinguish between subtypes of the α_{1A}-adrenoceptor.

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