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Pharmacological analysis of postjunctional α-adrenoceptors mediating contractions to (−)-noradrenaline in the rabbit isolated lateral saphenous vein can be explained by interacting responses to simultaneous activation of α_1-and α_2-adrenoceptors

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1 The pharmacological characteristics of the α-adrenoceptor population in the rabbit isolated saphenous vein has been examined with (−)-noradrenaline (NA), as principal agonist, and a number of antagonists with selectivity for either α_1- or α_2-adrenoceptors.

2 The rank order of potency of various agonists is consistent with a population of α_2-adrenoceptors; UK-14304 > (−)-noradrenaline = (−)-adrenaline > B-HT 920 = cirazoline > phenylephrine > amidephrine, but the rank order of pA_2 values for the antagonists against (−)-noradrenaline: BDF-6143 > rauwolscine = prazosin > CH-38083 = YM-12617 > Wy-26703 = phentolamine > corynanthine, is indicative of a mixed population of α_1- and α_2-adrenoceptors or, alternatively, a new subtype with characteristics of both the α_1- and α_2-subtypes.

3 Further evidence for two discrete populations of α-adrenoceptors is provided by, (a) the potent but non-competitive effect of prazosin against (−)-noradrenaline, (b) the presence of a component of the contractions elicited by NA and phenylephrine which is resistant to the selective α_2-adrenoceptor antagonists rauwolscine and CH-38083: these responses were inhibited by the selective α_1-adrenoceptor antagonists prazosin and YM-12617, but not by the selective α_2-adrenoceptor antagonist BDF-6143 and, (c) the relative potency of the yohimbine diastereoisomers rauwolscine and corynanthine against NA, phenylephrine and UK-14304.

4 In spite of the overwhelming evidence for a population of postjunctional α_2-adrenoceptors, prazosin was similarly effective against all agonists and failed to discriminate between those with putative selectivity for α_1- and α_2-adrenoceptors. This suggests an interaction of the effects of agonists at the two α-adrenoceptor subtypes.

5 An attempt has been made to reconcile a number of paradoxical observations with regard to the identification of postjunctional α_2-adrenoceptors *in vitro*, and it is suggested that in many of the isolated blood vessels presently available for examination both subtypes reside on the same smooth muscle cell. The pharmacological consequences of multiple subtypes of receptors mediating the same response is considered.

Introduction

The postjunctional location of both α_1- and α_2-adrenoceptor subtypes on vascular smooth muscle has been confirmed in many species *in vivo* (McGrath, 1982). Much of the available evidence for this sub-classification has been obtained from models in which pressor responses to various agonists, with known selectivity for prejunctional α_2-adrenoceptors, were resistant to the selective α_1-adrenoceptor antagonist prazosin (Cambridge *et al.*, 1977) but sensitive to the selective α_2-adrenoceptor antagonists yohimbine and rauwolscine (Weitzell *et al.*, 1979). In marked contrast, however, demonstration of both postjunctional subtypes *in vitro* has been much more difficult (McGrath, 1982; Hieble *et al.*, 1986).

In the course of our investigation into the pharmacological characteristics of postjunctional α-adrenoceptors in isolated blood vessels from the

rabbit (Daly et al., 1988b), we noted that insensitivity of (−)-noradrenaline (NA)-induced contractions to prazosin was an acceptable indicator for the presence of postjunctional α_2-adrenoceptors in two preparations (ear vein, plantaris vein). However, in the isolated lateral saphenous vein there was no 'prazosin-resistant' component of responses to NA, in spite of two other observations which could be taken to indicate the presence of postjunctional α_2-adrenoceptors; a comparison of the potency order of various 'selective' agonists and the relative potency of the yohimbine diastereoisomers rauwolscine and corynanthine.

This observation is very similar to that obtained in two earlier studies on the lateral saphenous vein (Purdy et al., 1980; Schümann & Lues, 1983), but conflicts with a more recent finding that this preparation possesses a population of postjunctional α_2-adrenoceptors that are insensitive to prazosin (Alabaster et al., 1985). Four possible explanatations that could reconcile these conflicting observations are as follows.

First, if both α_1- and α_2-adrenoceptors are present in the lateral saphenous vein, the use of a non-selective agonist (NA–Purdy et al., 1980; Daly et al., 1988b) precludes the possibility of detecting a prazosin-resistant response. Secondly, that both subtypes are present and responses mediated by the α_2-subtype are facilitated by the α_1-subtype. Thirdly, that the α-adrenoceptor population in this preparation is a functional example of the α_2-adrenoceptor binding site that prazosin binds to with high affinity (see: Neylon & Summers, 1985; Bylund, 1985; Kaposci et al., 1987). Fourthly, the different groups could be dealing with preparations that are indeed different because they have been taken from anatomically different regions of the vein, or because of inter-species variation.

We have attempted, therefore, to re-examine the population of postjunctional α-adrenoceptors in the lateral saphenous vein by employing NA as our principal agonist and various other agonists and antagonists selective for α_1- and α_2-subtypes. The results not only help to clarify the nature of the α-adrenoceptor population in this particular vein, but illustrate the problems inherent in analysing such a mixed population and point to some interaction between vascular responses mediated by receptor subtypes, which may be of more general applicability.

Methods

Tissue preparation

Albino New Zealand rabbits weighing between 2.3 kg and 3 kg were killed by stunning followed by exsanguination. The lateral saphenous veins in both legs were cleaned of fat and connective tissue *in situ* and then placed in ice-cold physiological salt solution (PSS). Four to six 3 mm long segments were taken from each vein and each segment was suspended between two wire supports as described by Hooker et al. (1976). The upper support was connected by cotton to a Grass FT03 transducer while the lower support was connected to a glass tissue holder. The preparations were then mounted in 30 ml organ baths under an initial resting tension of 2.0 g and allowed to relax. The final resting tension achieved on each segment varied between 0.3–0.5 g. Each preparation was bathed in PSS maintained at 37°C and gassed with 95% O_2 plus 5% CO_2.

Experimental procedure

After 60 min of equilibration each preparation was exposed to 3 μM (−)-noradrenaline (NA) and allowed to contract for 10 min. Following complete washout, an additional one hour equilibration period was allowed before commencing the experiment. This procedure was found to minimize changes in the sensitivity of the preparation to further addition of agonists, and is similar to the method used by Ruffolo et al. (1979). Contractions were recorded by means of a Grass FT03 transducer connected to a Linseis 6025 pen recorder.

In all experiments cumulative concentration-response curves (CRC) were determined by plotting the contractile response of the tissue to increasing concentrations (0.5 log unit increments) of the agonist. For the majority of preparations, contractile responses to the α-adrenoceptor agonists were not well maintained over a period greater than 90 s. Therefore, the addition of the next concentration of the agonist was made as close to the peak as possible. Successive CRC were separated by 60 min, as measured from the time following washout and complete relaxation of the preparation.

The following experiments were performed: (i) Agonists were compared with NA on the basis of potency ($pD_2 = -\log EC_{50}$ = the negative logarithm of the concentration required to produce 50% of maximum response interpolated from the CRC) and the maximum response relative to NA (E_{max}). No more than three agonists, including NA, were tested in any preparation.

(ii) Antagonists were added at least 40 min before the construction of a second or third CRC. For each curve the EC_{50} of the agonist was determined by interpolation. The agonist concentration-ratio (i.e. EC_{50} of the agonist in the presence of the antagonist divided by the control EC_{50} value) produced by the antagonist was determined at different concentrations spanning a range of 100 to 600 fold. According

to Arunlakshana & Schild (1959) if antagonism is competitive, a plot of the logarithm of (concentration-ratio −1) against the negative logarithm of the molar concentration of the antagonist yields a straight line whose slope is 1 and the intercept along the abscissa scale is the pA_2, which is equal to the antagonist dissociation constant (K_B) under equilibrium conditions. In all experiments, one preparation was run in parallel with experimental tissues, but received no antagonist, and was used to determine time-dependent changes in agonist sensitivity (Furchgott, 1972).

In some experiments, the agonist concentration-ratio values in the presence of rauwolscine alone and in combination with prazosin were determined at the 25%, 50% and 75% level of the maximum response to NA as described by Flavahan & Vanhoutte (1986). In addition, the negative logarithm of the dissociation constant ($-\log K_B$) for rauwolscine was also determined at each concentration by the agonist concentration-ratio method of Furchgott (1972).

All responses are expressed as a percentage of the maximum response and given as the mean ± s.e.mean. Differences between means were considered statistically significant if $P < 0.05$ for unpaired or paired observations (Student's t test).

Solutions and drugs

The composition of the PSS was (in mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 11. Na_2EDTA (23 μM) was also included in all experiments to prevent the oxidative degradation of NA and, unless indicated otherwise, propranolol (1 μM) and cocaine hydrochloride (10 μM) were also included to inhibit β-adrenoceptors and neuronal uptake of NA, respectively.

The following compounds were used: prazosin HCl (Pfizer); corynanthine HCl (Roth); rauwolscine HCl (Roth); Wy-26703 (N-methyl-N-(1,3,4,6,7,11b-hexahydro-2H-benzo-[a]-quinolizin-2-yl)-i-butane-sulphonamide HCl, Wyeth); YM-12617 (5-[2-[[2-(ethoxyphenoxy) ethyl] amino] propyl] - 2 - methoxy benzene-sulphonamide HCl, Yamanouchi); CH-38083 (7,8-(methylenedioxi)-14-α-hydroalloberbane HCl, Chinoin); BDF-6143 (4-chloro-2-(2-imidazolin-2-ylamino)-isoindoline (HCl, Biersdorf AG, Hamburg, F.R.G.); desipramine HCl (Sigma); normetanephrine HCl (Sigma); (−)-adrenaline bitartrate (Sigma); (−)-amidephrine mesyslate (Mead Johnson); cirazoline HCl (Synthelabo, Paris, France); B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-d]azepin-dihydrochloride, Boehringer Ingelhiem); UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate, Pfizer); (−)-phenylephrine HCl (Sigma); (−)-noradrenaline bitartrate (Sigma); propranolol HCl (Sigma); apo-yohimbine (Roth) and cocaine HCl (MacCarthys).

Results

The effects of neuronal uptake, extraneuronal uptake and β-adrenoceptor activation on the sensitvity of the saphenous vein to the contractile effects of NA

NA elicited concentration-related contractions in the rabbit isolated saphenous vein with a maximum response of 4.53 ± 0.04 g ($n = 6$).

Following the inclusion of the neuronal uptake inhibitor cocaine (10 μM) the NA CRC was significantly shifted leftwards (0.50 ± 0.08 of a log unit, $n = 6$, Table 1a). Similarly, the inclusion of the extraneuronal uptake inhibitor normetanephrine (30 μM) was associated with a small but significant leftward shift in the NA CRC (0.10 ± 0.04 of a log unit, $n = 10$). In view of the small magnitude of this shift, the importance of extraneuronal uptake was also assessed following α-adrenoceptor inhibition by various concentrations of phentolamine. Normetanephrine (30 μM) failed to alter significantly the slope of the Schild plot and this is taken as evidence that the extraneuronal route for the elimination of NA is of minor importance in this preparation (Table 1b). Preliminary experiments established that in preparations pretreated with 3 μM phenoxybenzamine for 30 min, 30 μM NA relaxed (72.2 ± 4.3%, $n = 4$) the vascular tone induced by 40 mM KCl and that this effect was abolished by 1 μM propranolol, indicating a β-adrenoceptor-mediated relaxant effect of high concentrations of NA. All subsequent experiments were performed in the presence of 10 μM cocaine and 1 μM propranolol only.

Rank order of agonist potency and E_{max} values

As shown in Table 2, the rank order of potency of the agonists based upon the pD_2 values was: UK-14304 = (−)-adrenaline = NA > B-HT 920 = cirazoline > phenylephrine > amidephrine.

Based upon the maximum contractions, NA, (−)-adrenaline and phenylephrine can be classed as full agonists, while UK = 14304, BHT-920, cirazoline and amidephrine are partial agonists compared with NA. An interesting feature of the agonism effected by both UK-14304 and cirazoline (not shown) was that the time to a steady response was longer than for responses elicited by the phenethylamine derivatives (Figure 1a).

The effects of various α-adrenoceptor antagonists on responses to NA

As shown in Figure 2 and Table 3, phentolamine

Table 1 (a) pD_2 values for noradrenaline (NA) in the rabbit isolated saphenous vein under various conditions. (b) pA_2 values and the slopes of the Schild plots for phentolamine against (−)-NA (with 95% confidence limits)

a *Agonist*	*pD_2*	*pD_2*	*log difference*
NA	6.89 ± 0.14	7.39 ± 0.07 Cocaine	0.50 ± 0.08*
NA Cocaine Propranolol	7.34 ± 0.07	7.44 ± 0.05 Cocaine Propranolol Normetanephrine	0.10 ± 0.04*

b	*pA_2*	*Slope*	*Conditions*
	7.24 (7.55–6.90)	0.86 (0.71–1.00)	Propanolol Cocaine
	7.46 (7.76–7.16)	0.87 (0.72–1.01)	Propranolol Cocaine Normetanephrine
	7.36 (7.53–7.15)	0.94 (0.82–1.06)	Cocaine Normetanephrine

(a) * Indicates $P < 0.05$ for 7–10 paired observations.
(b) $n = 18$ individual observations.
Concentrations employed: cocaine, 10 μM; propranolol, 1 μM; normetanephrine, 30 μM.

produced a competitive inhibition of responses to NA. There was a parallel rightward displacement of the CRC without a significant change in the maximum response and the slope of the Schild plot was not significantly different from unity. Similar effects were produced by BDF-6143 and corynanthine (Table 3).

Prazosin, Wy-26703 and YM-12617 caused concentration-dependent rightward parallel displacements of the NA CRC, but the slope of the Schild plot for each antagonist was significantly different from unity (Figure 2b and c, Table 3). A detailed examination of the Schild analysis for prazosin failed to reveal any differences in the slope of the plot when examined over a 20 fold, 60 fold and 600 fold concentration range (Figure 3).

In marked contrast, however, increasing concentrations of rauwolscine produced a non-parallel displacement of the NA CRC (Fig. 2d). A component of the response to NA, equivalent to approximately 20–35% of the maximum, was resistant to rauwolscine. An interesting feature of this response to

Table 2 pD_2 values with 95% confidence limits and E_{max} values for various agonists in the rabbit isolated saphenous vein in the presence of 10 μM cocaine and 1 μM propranolol

Agonist	*pD_2*	E_{max}	*pD_2 NA − pD_2 agonist*
NA ($n = 8$)	7.53 ± 0.08	1	
Adrenaline ($n = 8$)	7.69 ± 0.13	1.08 ± 0.03	−0.16
UK-14304 ($n = 8$)	7.85 ± 0.16	0.86 ± 0.04	−0.32
Cirazoline ($n = 9$)	6.54 ± 0.13	0.73 ± 0.04	1.01
B-HT 920 ($n = 6$)	6.41 ± 0.11	0.48 ± 0.04	1.12
Phenylephrine ($n = 7$)	5.83 ± 0.06	0.95 ± 0.02	1.70
Amidephrine ($n = 4$)	5.27 ± 0.08	0.61 ± 0.03	2.28

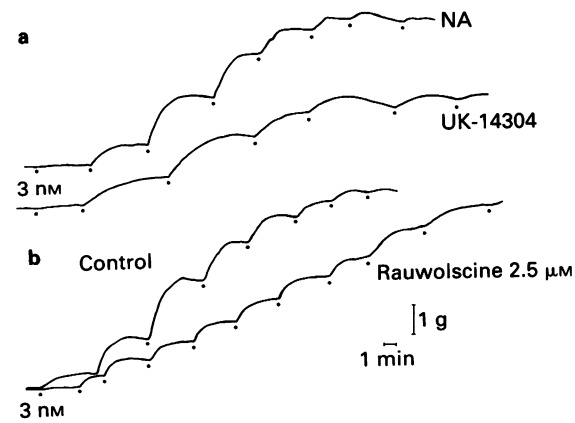

Figure 1 Representative trace recordings of cumulative responses to noradrenaline (NA) and UK-14304 in the rabbit isolated saphenous vein (a) and of cumulative responses to NA elicited in the absence and presence of rauwolscine 2.5 μM (b).

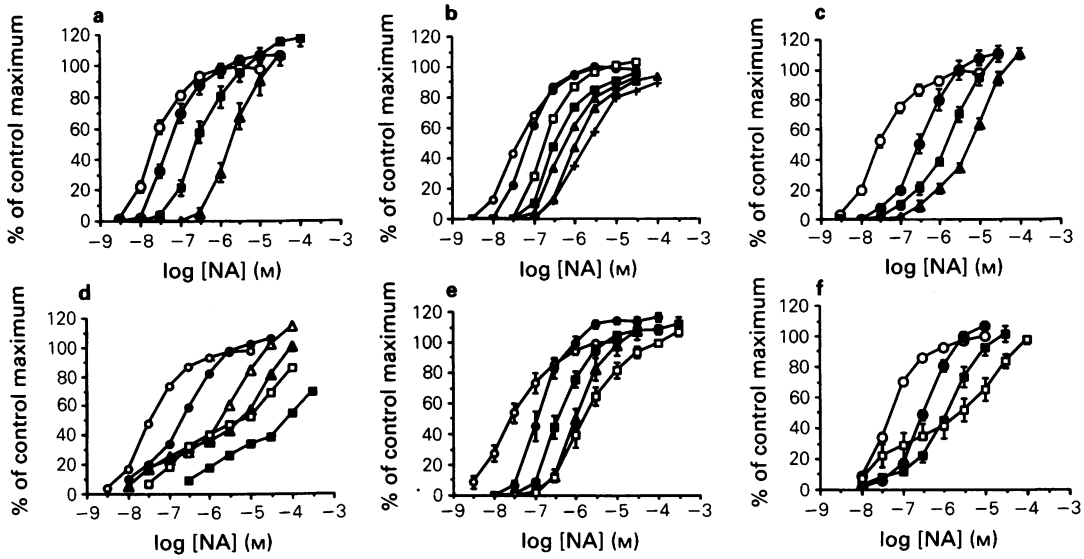

Figure 2 The effects of various α-adrenoceptor antagonists on contractile responses to noradrenaline (NA) in the rabbit isolated saphenous vein; (a) 0.05 μM (●), 0.5 μM (■) and 5 μM (▲) phentolamine: (b) 0.005 μM (●), 0.03 μM (□), 0.1 μM (■), 0.3 μM (△), 1 μM (▲) and 3 μM (+)-prazosin; (c) 0.3 μM (●), 3 μM (■), 15 μM (▲) Wy-26703; (d) 0.05 μM (●), 0.5 μM (△), 2.5 μM (▲), 12.5 μM (□) and 50 μM (■) rauwolscine; (e) 0.5 μM (●), 2.5 μM (■), 12.5 μM (▲) and 50 μM (□) corynanthine; (f) 0.1 μM (●), 1 μM (■) and 10 μM (□) CH-38083. The control CRC to NA on each graph is represented by (○). All points represent the mean of 4–9 observations from different animals and the vertical lines, where shown, indicate the s.e.mean. In (b) and (d) s.e.mean values have been omitted to improve clarity and these were always less than ±7%.

NA was the very rapid time to peak response compared with that in the absence of the antagonist (Figure 1b). Quantitatively similar results were observed for rauwolscine when cocaine was replaced with 0.1 μM desipramine. Similarly, both CH-38083 (Figure 2f) and apo-yohimbinine (2.5 μM) effected a non-parallel displacement of the NA CRC. In the case of CH-38083, the resistant component was more pronounced with the highest concentration of the antagonist (10 μM) than was observed with lower concentrations. Based upon a Schild plot of the agonist concentration-ratio at the 75% level of the maximum response, both rauwolscine and CH-38083 inhibited responses in a non-competitive manner (Table 3).

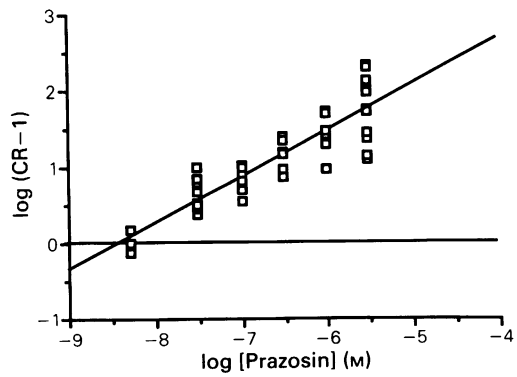

Figure 3 Schild analysis for the effect of prazosin (0.005 μM–3 μM) on contractile responses to noradrenaline (NA) in the rabbit isolated saphenous vein. Log agonist concentration-ratio (CR) values produced by prazosin were determined at the 50% level of the control maximum and a minimum number of 5 determinations was made at each antagonist concentration.

As shown in Table 4, the agonist concentration-ratios calculated at the level of 25% and 75% of maximum for NA in the presence of 2.5 μM rauwolscine were significantly different from each other; further evidence for the non-parallel displacement of the CRC. Interestingly, in approximately 30% of the preparations the addition of rauwolscine to the bathing medium resulted in a small contraction (<10% of the maximum response to NA) which invariably returned to baseline tension over 15 min. Rauwolscine (0.5–12.5 μM), corynanthine (50 μM) and, less frequently, phentolamine (0.5–5 μM) were the only antagonists that produced contractions and these were abolished by the presence of prazosin (0.1 μM), YM-12617 (0.1 μM), corynanthine (2.5 and 12.5 μM) or phenoxybenzamine (0.03 μM). Neither CH-38083 (0.1–10 μM) nor BDF-6143 (5 nM–0.5 μM)

Table 3 pA_2 values and the slopes of the Schild plots (with 95% confidence limits) for various antagonists against NA-induced contractions of the rabbit isolated saphenous vein.

Antagonists	*pA_2*	*Slope*
BDF-6143	8.74	0.88
(0.005–0.5 μM)	(8.87–8.51)	(0.75–1.02)
Rauwolscine	8.56	0.85
(0.05–2.5 μM)	(8.89–8.22)	(0.74–0.96)*
Prazosin	8.44	0.58
(0.005–3 μM)	(8.88–8.11)	(0.47–0.69)*
YM 12617	8.06	0.69
(0.01–1.0 μM)	(8.60–7.60)	(0.46–0.92)*
CH-38083	8.00	0.68
(0.1–10 μM)	(8.69–7.31)	(0.48–0.87)*
Wy-26703	7.70	0.80
(0.3–15 μM)	(8.04–7.36)	(0.68–0.92)*
Phentolamine	7.24	0.86
(0.05–5 μM)	(7.55–6.90)	(0.71–1.00)
Corynanthine	6.36	0.89
(0.5–50 μM)	(6.55–6.16)	(0.71–1.06)

pA_2 values were determined from a regression analysis of the log agonist concentration-ratio from 15–56 individual observations with a minimum of 4 points determined at 3–5 different concentrations.
pA_2 values for rauwolscine and CH-38083 were determined from the agonist concentration-ratio at the 75% level of the maximum response.
* Slope of Schild plot significantly different from 1.

Table 4 Log agonist concentration-ratios for noradrenaline (NA) measured at the 25%, 50% and 75% of maximum for NA in the presence of 0.1 μM prazosin and various concentrations of rauwolscine and different combinations of both antagonists

Antagonists	*25%*	*50%*	*75%*
Prazosin 0.1 μM	1.12 ±0.06	1.01 ±0.04	1.07 ±0.08
Rauwolscine 0.05 μM	0.67 ±0.04	0.93 ±0.01	1.07 ±0.02
Rauwolscine 0.05 μM + prazosin 0.1 μM	1.69* ±0.08	1.65* ±0.09	1.51* ±0.09
Rauwolscine 0.5 μM	1.01 ±0.40	1.39 ±0.42	1.86 ±0.19
Rauwolscine 0.5 μM + prazosin 0.1 μM	1.99* ±0.14	1.98 ±0.12	1.86 ±0.17
Rauwolscine 2.5 μM	1.37 ±0.32	1.79 ±0.26	2.35 ±0.11
Rauwolscine 2.5 μM + prazosin 0.1 μM	2.42* ±0.24	2.53* ±0.18	2.45 ±0.11

* Indicates a significant increase in the agonist concentration-ratio in the presence of prazosin. n = 5–9 observations.

elicited contractions of the saphenous vein, while 15 nM BDF-6143 did not reduce transient responses to rauwolscine (CH-38083 was not tested against rauwolscine). All of these observations suggest that the contractile effect of the antagonists is due to activation of an α_1-adrenoceptor rather than an α_2-adrenoceptor.

Based upon the pA_2 values for the antagonists (Table 3), the rank order of potency versus NA was: BDF-6143 > rauwolscine = prazosin > YM-12617 = CH-38083 > Wyeth 26703 = phentolamine > corynanthine.

A comparison of the pA_2 values for these antagonists against NA in the rabbit isolated saphenous vein and values observed at α-adrenoceptor subtypes in the rat and rabbit is shown in Figure 8.

The effects of various antagonists on the 'rauwolscine-resistant' component of responses to NA

The effects of prazosin (0.1 μM) on responses to NA in the presence of various concentrations of rauwolscine (0.05–2.5 μM) are shown in Figure 4, and the effects of corynanthine (2.5 μM), YM-12617 (0.1 μM) or BDF 6143 (15 nM) in combination with rauwolscine (2.5 μM) are shown in Figure 5. The basis of the choice of these antagonist concentrations was that they produced a 5–10 fold parallel shift of the NA CRC. Both prazosin and YM-12617 abolished the 'rauwolscine-resistant' component to NA but corynanthine and BDF 6143 did not. Prazosin (0.1 μM) was also able to abolish the component of the NA CRC that was 'resistant' to CH-38083 (Figure 5d).

The interaction between prazosin (0.1 μM) and rauwolscine (0.05 μM, 0.5 μM and 2.5 μM) was examined in greater detail (Table 4 and Figure 4). For each concentration of rauwolscine, with the exception of 0.05 μM rauwolscine, prazosin increased the agonist concentration-ratio at the 25% level of maximum without significantly increasing the agonist concentration-ratio measured at the 75% of maximum level, thereby reducing the difference between these two values and increasing the slope of the CRC. However, even in the presence of 0.1 μM prazosin, $-\log K_B$ values for 0.05 μM and 0.5 μM rauwolscine were significantly different (determined in paired preparations at the level of the 50% of maximum, $-\log K_B$ values were 7.90 ± 0.04 (n = 3) and 7.56 ± 0.09 (n = 3), respectively). This indicates that, although 0.1 μM prazosin can abolish the 'rauwolscine-resistant' component of NA responses, the action of rauwolscine on the remaining contractile responses still cannot be described as competitive.

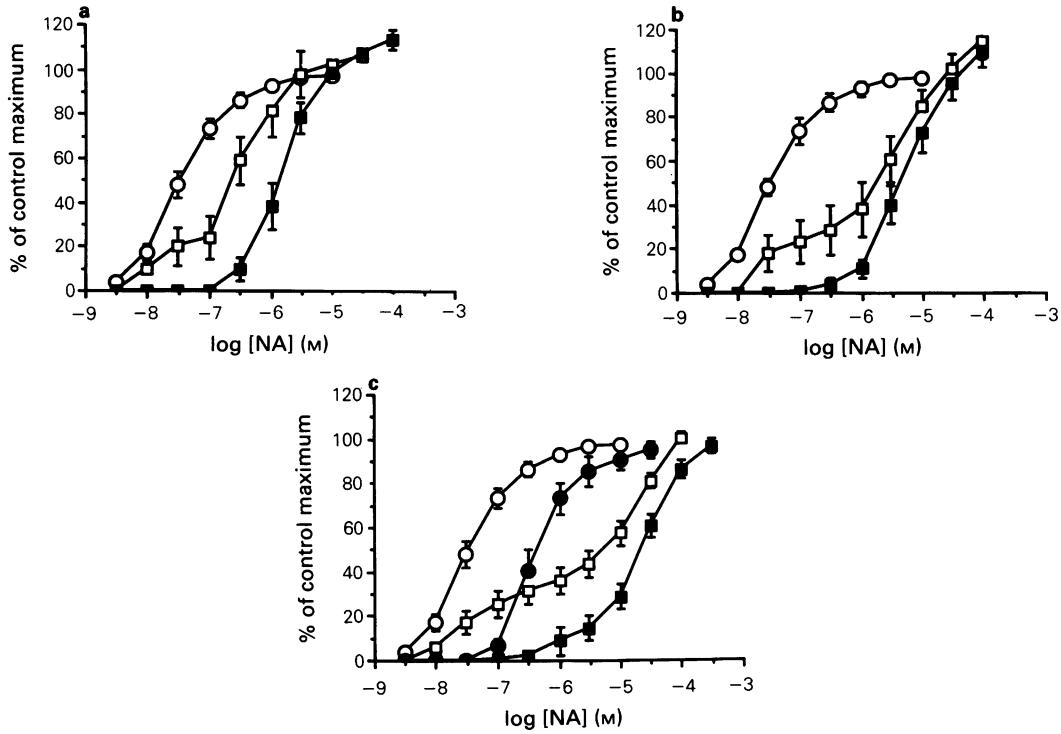

Figure 4 The effect of 0.05 μM (a), 0.5 μM (b) and 2.5 μM (c) rauwolscine on contractile responses to noradrenaline (NA) in the absence (□) and in the presence of 0.1 μM prazosin (■). The control concentration-response curve to NA on each graph is represented by (○) and the effects of 0.1 μM prazosin alone on responses to NA are shown in (c) as (●). All points represent the mean of 5–9 observations from different animals and the vertical lines indicate the s.e.mean.

The effects of rauwolscine, corynanthine and prazosin on responses to UK-14304 and phenylephrine

Rauwolscine was 20 to 100 fold more potent than corynanthine against either agonist (Figure 6a and c). However, a feature of the inhibition produced by rauwolscine against phenylephrine, but not UK-14304, was that the highest concentration (2.5 μM) significantly reduced the maximum response and revealed a 'resistant' component. In contrast, inhibition by corynanthine of responses to both agonists was not associated with either a resistant component or a reduction in the maximum response.

Prazosin (0.1 μM and 3 μM) produced concentration-related inhibition of responses to phenylephrine (Figure 6d), but the agonist concentration-ratio values were significantly smaller than those produced against NA (Table 5). For UK-14304, responses to all concentrations were reduced by prazosin (0.03–3 μM) but those elicited by high concentrations (> 1 μM) were more affected (Figure 6b). A feature of the responses to high concentrations of UK-14304 in the presence of prazosin was that they were poorly maintained (sometimes returning back to baseline) and the preparation sometimes became completely unresponsive to further cumulative addition of the agonist (Figure 7). This effect was more pronounced with higher concentrations of prazosin (> 0.3 μM) and, thus, renders impossible any determination of a pA_2 value or log agonist concentration-ratio from the UK-14304 CRC.

Discussion

Overall, the potencies of the agonists in this preparation strongly indicate a predominance of α_2-adrenoceptors over α_1-adrenoceptors mediating contractile responses. Antagonist potency does not, however, give clear support for this since high pA_2 values were obtained against NA for several agents which, in other preparations, are highly selective for

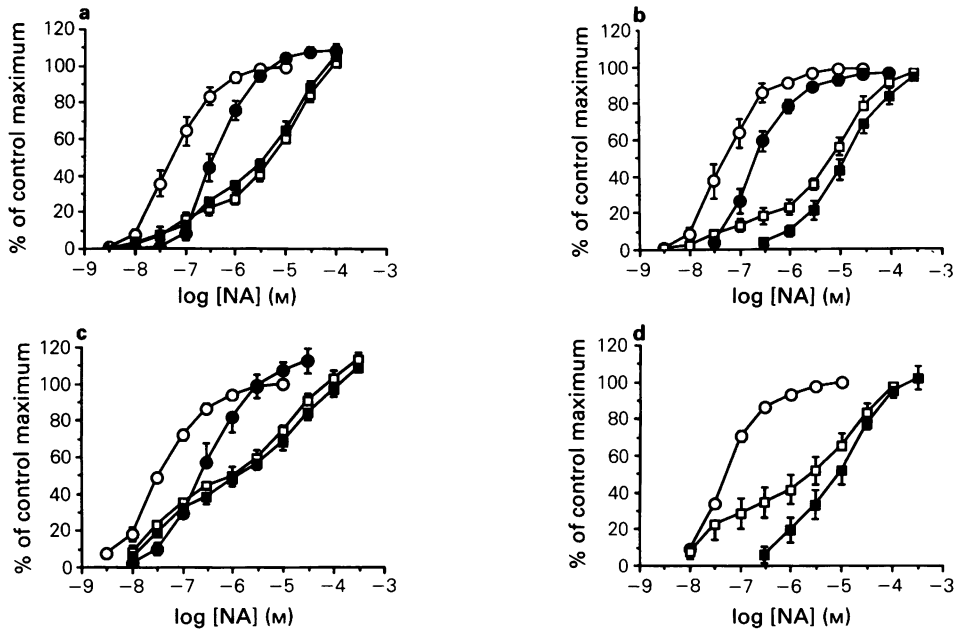

Figure 5 A comparison of the effect of several α-adrenoceptor antagonists on the 'resistant' component of responses to noradrenaline (NA) elicited in the presence of either rauwolscine or CH-38083: (a) 2.5 μM corynanthine alone (●) and in the presence of 2.5 μM rauwolscine (■); (b) 0.1 μM YM-12617 alone (●) and in the presence of 2.5 μM rauwolscine (■); (c) 0.015 μM BDF-6143 alone (●) and in the presence of 2.5 μM rauwolscine (■); (d) 10 μM CH-38083 alone (□) and in the presence of 0.1 μM prazosin (■). The control responses to NA on each graph are represented by (○) and the effect of either 2.5 μM rauwolscine or 10 μM CH-38083 alone is shown as (□). All points represent the mean of 5–7 observations from different animals and the vertical lines indicate the s.e.mean.

either α_1- or α_2-adrenoceptors. This points to either an unconventional α-adrenoceptor or to a mixed population of α_1- and α_2-adrenoceptors. We favour the latter.

Evidence for two populations of α-adrenoceptors

A feature of the inhibition produced by most antagonists against NA-induced contractions was the tendency for the slope of the Schild plot to be 0.9 or less, even though the 95% confidence limits often overlapped unity. It seems unlikely that this apparent deviation from unity is attributable to incomplete inhibition of neuronal uptake, as the effect of 10 μM cocaine on the CRC to NA was not significantly different from that produced by 30 μM cocaine (results not shown). Nor does it seem likely that the decision to leave extraneuronal uptake intact is a

Table 5 Log agonist concentration-ratios produced by 0.1 μM and 3 μM prazosin, 0.5 μM corynanthine and 0.5 μM rauwolscine in the rabbit isolated saphenous vein

Antagonists	*Noradrenaline*	*Phenylephrine*	*UK-14304*
Prazosin 0.1 μM	1.01 ±0.04	0.51 ±0.17	
Prazosin 3 μM	1.75 ±0.17	1.04 ±0.20	
Rauwolscine 0.5 μM	1.39 ±0.42	1.75 ±0.14	1.42 ±0.09
Corynanthine 0.5 μM	0.40 ±0.05	0.21 ±0.24	0.48 ±0.11

Values were determined at the 50% of the maximum response produced by the agonist. n = 4–6 observations.

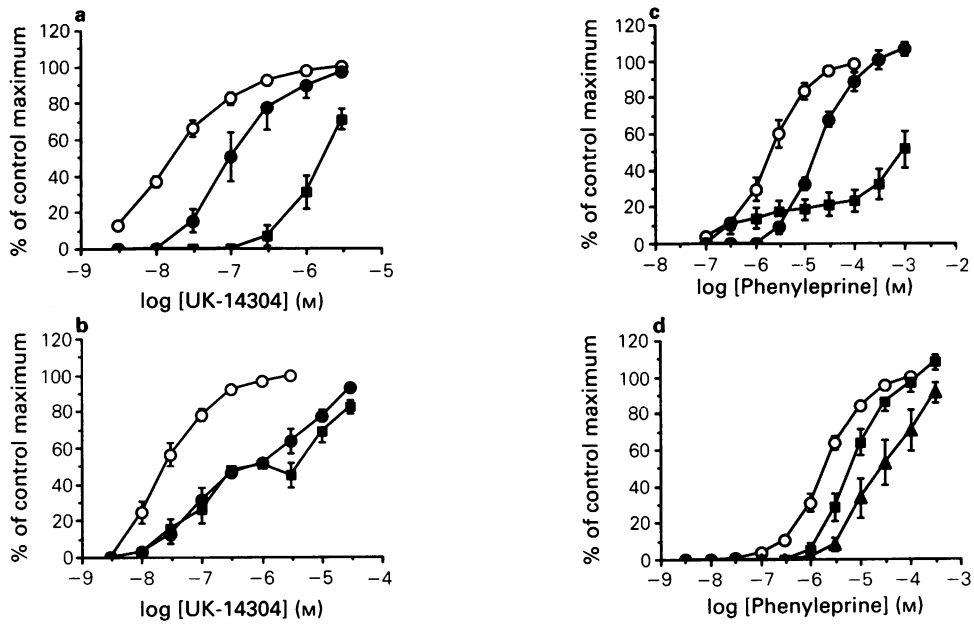

Figure 6 The effect of corynanthine, rauwolscine and prazosin on contractions elicited by phenylephrine and UK-14304 in the rabbit isolated saphenous vein: (a) responses to UK-14304 (○) elicited in the presence of corynanthine 2.5 μM (●) and rauwolscine 2.5 μM (■); (b) responses to UK-14304 (○) elicited in the presence of prazosin 0.03 μM (●) and 0.1 μM (■); (c) responses to phenylephrine (○) elicited in the presence of corynanthine 2.5 μM (●) and rauwolscine 2.5 μM (■); (d) responses to phenylephrine (○) elicited in the presence of prazosin 0.1 μM (■) and 3 μM (▲). All points represent the mean of 5–7 observations from different animals and the vertical lines indicate the s.e.mean.

possible explanation, since the slope of the Schild plot for phentolamine, an antagonist which does not distinguish between α_1- and α_2-adrenoceptors (McGrath, 1982), was not influenced by the inclusion of the extraneuronal uptake inhibitor, normetanephrine (see: Furchgott, 1972). Since the antagonists employed are known to possess varying degrees of selectivity for α_1- and α_2-adrenoceptors (see: Table 6; Figure 8), the most plausible explanation is the presence of two populations of α-adrenoceptors. This view was endorsed by several observations.

(1) The rank order of pA_2 values for the antagonists does not bear any relationship to the action of NA at a single population of postjunctional α_1- or α_2-adrenoceptors. Prazosin and YM-12617, both selective for α_1-adrenoceptors (Cambridge *et al.*, 1977; Honda *et al.*, 1985; Figure 8), inhibited responses at concentrations well below those shown to be effective at α_2-adrenoceptors and were as potent as the selective α_2-adrenoceptor antagonists Wy-26703 and CH-38083. For YM-12617, however, this concentration was also higher (10 nM) than that needed for an effect at α_1-adrenoceptors in the rat and the rabbit (0.1–1 nM – see Figure 8). In marked contrast, BDF-6143 and rauwolscine were the most potent antagonists with activities consistent with an effect at α_2-adrenoceptors (Weitzell *et al.*, 1979; Docherty *et al.*, 1982). These seemingly contradictory observations can be reconciled by assuming the presence of both subtypes, with the α_2-adrenoceptor predominating. This would explain why the pA_2 values for α_2-selective antagonists approximate to their known value at α_2-adrenoceptors, while pA_2 values for the selective α_1-adrenoceptor antagonists are either intermediate between their potency at α_1- and α_2-adrenoceptors or at the low end of their

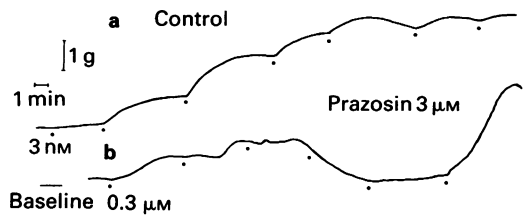

Figure 7 Representative recordings of cumulative concentration-responses to UK-14304 in the rabbit isolated saphenous vein in the absence (a) and presence (b) of prazosin.

Table 6 A comparison of pA_2 values for various antagonists at pre- and post-junctional α_1- and α_2-adrenoceptors in isolated preparations from the rabbit and the rat with values obtained against noradrenaline (NA) in the rabbit isolated saphenous vein (RLSV)

Antagonists	*Type*	*Preparation*	*pA_2*	*α_1/α_2*	*RLSV pA_2*
Prazosin	Post-α_2	Rabbit saphenous vein	6.8 (l)		
	Pre-α_2	Rat vas deferens	5.16 (a)		
	Pre-α_2	Rabbit vas deferens	<5.0 (g)		
	Pre-α_2	Rabbit pul' artery	6.3 (k)	200–3000	8.44
	Post-α_1	Rabbit aorta	8.85 (a)	from rabbit to	
	Post-α_1	Rabbit ear artery	8.19 (b)	rat	
	Post-α_1	Rabbit pul' artery	8.76 (c)		
	Post-α_1	Rat aorta	10.0 (f)		
	Post-α_1	Rat portal vein	9.3 (f)		
YM-12617	Pre-α_2	Rat vas deferens	6.4 (a)		
	Post-α_1	Rabbit aorta	10.0 (a)	3000	8.06
	Post-α_1	Rat aorta	9.9 (f)		
Corynanthine	Pre-α_2	Rat vas deferens	5.0 (j)		
	Pre-α_2	Rabbit pul' artery	5.0 (e)*		
	Post-α_2	Rabbit ear vein	6.22 (d)	5–10	6.36
	Post-α_1	Rat anococcygeus	7.3 (j)		
	Post-α_1	Rabbit aorta	7.06 (d)		
	Post-α_1	Rabbit pul' artery	6.6 (e)		
Phentolamine	Pre-α_2	Rat vas deferens	8.02 (c)	1	7.24
	Post-α_1	Rabbit pul' artery	7.82 (c)		
	Post-α_1	Rabbit ear artery	7.8 (b)		
Wy-26703	Pre-α_2	Rat vas deferens	8.16 (g)		
	Pre-α_2	Rabbit vas deferens	6.43 (g)		
	Post-α_2	Rabbit ear vein	7.3 (d)	1–0.1	7.7
	Post-α_2	Rabbit saphenous vein	6.25 (i)		
	Post-α_1	Rat anococcygeus	6.49 (g)		
BDF-6143	Post-α_1	Rabbit pul' artery	7.38 (h)		
	Post-α_1	Rabbit aorta	7.09 (h)	0.1	8.74
	Pre-α_2	Rabbit pul' artery	8.28 (h)		
Rauwolscine	Pre-α_2	Rat anococcygeus	7.5 (j)		
	Pre-α_2	Rabbit vas deferens	8.1 (g)		
	Post-α_2	Rabbit ear vein	7.7 (d)	0.02–0.002	8.56
	Post-α_2	Rabbit saphenous vein	8.5 (l)		
	Post-α_1	Rabbit ear artery	5.38 (b)		
	Post-α_1	Rabbit pul' artery	5.89 (e)		
	Post-α_1	Rabbit aorta	5.8 (e)		
CH-38083	Pre-α_2	Rat vas deferens	8.30 (c)	0.01–0.002	8
	Post-α_1	Rabbit pul' artery	5.55 (c)		
	Post-α_2	Rabbit ear vein	7.7 (d)		

Where possible the α_1/α_2 ratio is derived from studies in the rabbit.

* Value estimated as the concentration required to increase the release of [^{3}H]-NA by 30%. (a) Honda *et al.*, (1985). (b) Hieble & Woodward (1984). (c) Vizi *et al.* (1986). (d) Daly *et al.* (1987b). (e) Weitzell *et al.* (1979). (f) unpublished observations. (g) Lattimer & Rhodes (1985). (h) Docherty *et al.* (1982). (i) Alabaster *et al.* (1986). (j) McGrath (1984). (k) Kaposci *et al.* (1987). (l) Schümann & Lues (1983).

activity at α_1-adrenoceptors(see Figure 8).

(2) Although increasing concentrations of prazosin caused a parallel displacement of the concentration-response curve for NA, the slope of the Schild plot was significantly less than unity. This finding agrees well with earlier results (Purdy *et al.*, 1980; Schümann & Lues, 1983). According to Milnor (1986), the concentration range of prazosin employed (600 fold) should have been sufficiently large for the Schild analysis to reveal the involvement of two receptors in responses to NA (as judged by two distinct components contributing to the overall slope of the

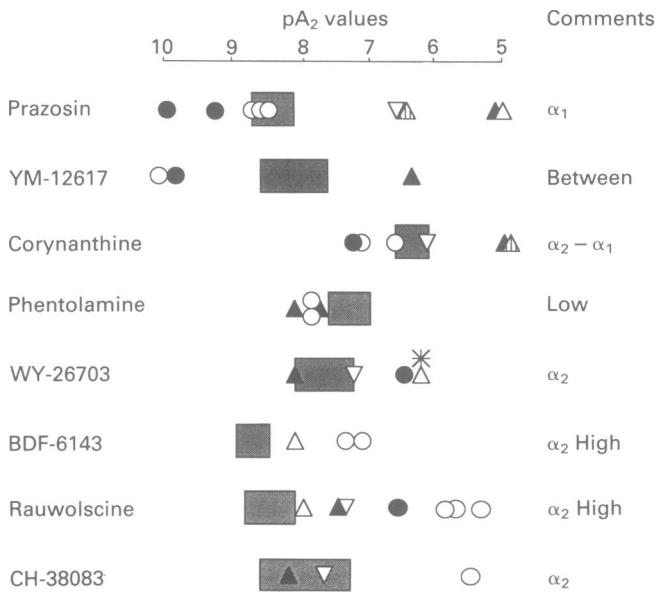

Figure 8 A comparison of the pA_2 values for various α-adrenoceptor antagonists against noradrenaline (NA)-induced contractions in the rabbit isolated saphenous vein (present study) with pA_2 values found previously at α_1- and α_2-adrenoceptors in the rabbit and the rat. pA_2 values and 95% confidence limits for antagonists from the present study are presented by (□); the centre of the box represents the pA_2 value and the width of the box represents the 95% confidence limits. Reported pA_2 values for the antagonist at post-α_1- (○), pre-α_2- (△) and post-α_2-adrenoceptors (▽) in the rat (closed symbols) and the rabbit (open symbols) are taken from the references cited in Table 6. (*) Represents a value determined in the rabbit isolated saphenous vein and the hatched triangle indicates that the potency of the antagonist at prejunctional α_2-adrenoceptors was estimated from the concentration required to increase the release of [^{3}H]-NA by 30%. The comments refer to the activity of the antagonist in the saphenous vein in relation to values found previously in isolated preparations in the rabbit (or where this is not available, in the rat).

Conclusion: All selective α_2-adrenoceptor antagonists and corynanthine fit with the view that the major subtype is an α_2-adrenoceptor. Phentolamine is less potent than would normally be expected for an α-adrenoceptor antagonist (pA_2 approx. 8), but this is not too unusual. Prazosin and YM-12617 lie 'in between' expected activity at either subtype, or at the low end of activity at α_1-adrenoceptors. It should be noted, however, that prazosin was *less* potent against the 'selective' α_1-adrenoceptor agonist phenylephrine than against NA.

Schild plot) if the lesser population accounts for more than 30% of the maximum response. Since the rauwolscine-sensitive component of responses to NA contributes to approxiamtely 65% of the maximum response this did not seem unreasonable. However, analysis of the agonist concentration-ratio over a 20 fold, 60 fold and 600 fold antagonist concentration range failed to reveal any differences in the slope (all were approximately 0.6). Although this could be interpreted as evidence for a non-competitive action of prazosin at a single population of α-adrenoceptors, the finding that the slope of the Schild plot for the structurally dissimilar selective α_1-adrenoceptor antagonist YM-12617 (Honda *et al.*, 1985) was also significantly less than unity is again consistent with the view that this is the result of the presence of two different populations of α-adrenoceptor.

(3) The selective α_2-adrenoceptor antagonists rauwolscine (Weitzell *et al.*, 1979) and CH-38083 (Vizi *et al.*, 1986) produced non-parallel displacements of the NA CRC. A component of the CRC, approximately 20–30% of the maximum, was resistant to rauwolscine and this necessitated determination of the agonist concentration-ratio values at the 75% level of the maximum response, rather than at the 50% level. The appearance of the rauwolscine-resistant component to NA was also observed in the absence of both cocaine and propranolol or in the presence of the neuronal uptake inhibitor desipramine, thus eliminating the possibility that this is due to an effect of the ancillary drugs used to optimize the condi-

tions for the study of the α-adrenoceptors (see Schümann & Lues, 1983). The appearance of a 'rauwolscine-resistant' response to NA could conceivably be a consequence of the transient contractile response to rauwolscine reducing the threshold for contraction (see: Stupecky *et al.*, 1986), but this seems unlikely since CH-38083 failed to elicit similar responses while corynanthine (50 μM) and phentolamine caused transient contractions without effecting a change in the slope of the NA CRC. Furthermore, transient contractions produced by prazosin in the rabbit isolated aorta (Cavero *et al.*, 1978) and yohimbine in the rabbit isolated ear artery (Tayo, 1982) were not associated with the appearance of a resistant component to NA.

It is noteworthy that the antagonists which produced changes in the slope of the NA CRC, rauwolscine and CH-38083, are not only the most selective for α_2-adrenoceptors (Table 6), but are also structurally related (Vizi *et al.*, 1986). This suggests that selectivity for the postjunctional α_2-subtype alone may be insufficient for an antagonist to 'unveil' the α_1-subtype. This is endorsed by two other observations. First, another structurally similar though less selective antagonist, apo-yohimbine (McGrath, 1984), has the ability to effect similar, though less pronounced, changes in the NA CRC. Secondly, there was no 'resistant' component to NA in the presence of the selective α_2-adrenoceptor antagonists Wy-26703 and BDF-6143 (Figure 2 and Table 6). Although the slope of the Schild plot for Wy-26703 was significantly less than unity, which supports the view that both α_1- and α_2-adrenoceptors are present, accurate assessment of its relative selectivity for the postjunctional α_2-adrenoceptor subtype compared with rauwolscine or apo-yohimbine is hindered by the lack of quantitative data at both postjunctional subtypes in isolated preparations from the rabbit (see Figure 2 and Table 6).

(4) Concentrations of the selective α_1-adrenoceptor antagonists prazosin and YM-12617 which produced a 5–10 fold shift of the NA CRC abolished the rauwolscine (2.5 μM) 'resistant' component of responses to NA, while similarly effective concentrations of BDF-6143 and corynanthine were unable to reduce this component. The lack of effect of corynanthine on the 'rauwolscine-resistant' response to NA appears to reflect the poor selectivity which this antagonist possesses for postjunctional α_1- and α_2-adrenoceptors in the rabbit, especially apparent between the venous α-adrenoceptor subtypes (Daly *et al.*, 1988b), and emphasizes that the discriminating power of these two diastereoisomers in the rabbit resides mainly with rauwolscine (Figure 8).

In spite of the apparent effectiveness of prazosin (0.1 μM) against the 'rauwolscine-resistant' component, which might be taken as evidence that an 'α_1-like' receptor had been completely inhibited, small differences in the $-\log K_B$ values for 0.05 μM and 0.5 μM rauwolscine (in the presence of prazosin) suggest that the remaining responses to NA may not be the result of activation of a single receptor. This illustrates the difficulty inherent in the routine use of competitive antagonists selective for one subtype when attempting to investigate the characteristics of another receptor subtype that mediates the same functional response.

(5) The rank order of potency of agonists in this preparation is similar to that found by Schümann & Lues, (1983) and Alabaster *et al.* (1985) and is consistent with the presence of predominantly the α_2-subtype. The selective α_2-adrenoceptor agonist UK-14304 (Cambridge, 1981) was equipotent with both NA and (−)-adrenaline and 100 fold more potent than the selective α_1-adrenoceptor agonists amidephrine and phenylephrine (McGrath, 1982). In addition, the selective α_2-adrenoceptor agonist B-HT 920, though less potent than both NA and UK-14304, was also more potent than the selective α_1-adrenoceptor agonists.

Evidence for the presence of two α-adrenoceptor subtypes, however, is provided by the ability of rauwolscine to inhibit the major component of the contractile response to phenylephrine and reveal a small 'resistant' component. Similar observations with this combination of agonist and antagonist were made by both Alabaster *et al.* (1985) and Schümann & Lues (1983) but were not discussed. Thus, in this preparation phenylephrine-induced contractions can be attributed mainly to an α_2-adrenoceptor population with a smaller component via a population of α_1-adrenoceptors.

The lack of specificity of various 'selective' adrenoceptor agonists in a preparation with a mixed population of receptor subtypes is further underlined by the finding that a major component of the contraction elicited by the selective α_2-adrenoceptor agonist UK-14304 is sensitive to a low concentration of prazosin (0.03 μM). However, the residual response to UK-14304 was not reduced further by increasing the prazosin concentration to 0.1 μM (Figure 7c) and, as such, represents the first clearcut example of a prazosin-resistant α_2-adrenoceptor-mediated response in this vessel. Interestingly, Gisclard *et al.* (1987) noted that in the presence of 0.1 μM prazosin, UK-14304 elicited concentration-dependent contractions of the rabbit isolated saphenous vein with a pD_2 of 7 and E_{max} of 0.4 (compared to NA); this is essentially the same as the results with 0.1 μM prazosin and UK-14304 in the present study. Attempts to quantify the effects of higher concentrations of prazosin were abandoned because 'responses' to further cumulative addition of the agonists were extremely variable and not maintained (Figure 7). The basis for

this effect of prazosin is not known, though similar difficulties have been encountered with prazosin and UK-14304 in the dog isolated saphenous vein (N.A. Flavahan, personal communication). It perhaps may be worthwhile investigating whether the same phenomenon can occur with non-cumulative CRC to UK-14304 in the presence of prazosin.

Prazosin also exerted a significant inhibition of contractions elicited by another selective α_2-adrenoceptor agonist B-HT-920 (Schümann & Lues, 1983; authors unpublished observation) which, like UK-14304, did not elicit a 'rauwolscine-resistant' response. In marked contrast, responses to phenylephrine were clearly more resistant to prasozin than were those to UK-14304; a surprising observation in view of their respective selectivity profiles.

Functional interaction between α-adrenoceptor subtypes?

Taken together, these observations lend support to the view that for the rabbit isolated saphenous vein contractions to all of the α-adrenoceptor agonists tested are mediated by a population of postjunctional α-adrenoceptors and, to a lesser extent, a populations of α-adrenoceptors in the rabbit isolated sapsimilar to those in the rabbit isolated ear vein and rabbit isolated plantaris vein (with the exception of the pronounced inhibitory effects of prazosin). However, the nature of the 'response' mediated by the α_1-adrenoceptors permits only a qualitative comparison with other populations of postjunctional α_1-adrenoceptors (see: Daly *et al.*, 1988b). Interestingly, the contractile effect of rauwolscine appears to be mediated by an 'α_1-like adrenoceptor' since it is sensitive to prazosin, YM-12617 and phenoxybenzamine, but not affected by BDF-6143. However, since contractile responses to rauwolscine were not observed in the thoracic aorta, ear artery or renal vein (Daly *et al.*, 1988b), this suggests that there may be subtle differences between the 'α_1'-adrenoceptors present in the saphenous vein and those elsewhere. Paradoxically, these observations also appear to reaffirm the selectivity of BDF-6143 for α_2-adrenoceptors (Docherty *et al.*, 1982), yet they fail to account for the absence of a resistant component of the responses to NA with this antagonist.

Acceptance of the presence of two discrete populations of α-adrenoceptor in the rabbit isolated saphenous vein eliminates the possibility that the sensitivity of NA responses to prazosin is the result of the presence of a single population of α-adrenoceptors which possess pharmacological characteristics common to both α_1- and α_2-adrenoceptors (cf: the prazosin-sensitive α_2-adrenoceptor described by Hieble & Woodward, 1984; Bylund, 1985; Neylon & Summers, 1985; Kaposci *et al.*, 1987). Furthermore, since responses to all agonists were inhibited by 0.1 μM prazosin, a concentration used routinely to effect selective blockade of α_1-adrenoceptors (Flavahan & Vanhoutte, 1986), the absence of a sizeable 'prazosin-resistant' α_2-adrenoceptor-mediated response to NA cannot be attributed solely to the non-selective nature of the catecholamine. Indeed, if the selectivity of prazosin remains unquestioned, the only satisfactory explanation for the observations herein is as follows.

(a) All agonists, irrespective of their reported selectivity, are capable of 'stimulating' both α_1- and α_2-adrenoceptors. In the case of agonists with known activity at α_1-adrenoceptors (e.g.: phenylephrine and NA), this gives rise to a contractile reponse resistant to rauwolscine, while agonists selective for α_2-adrenoceptors (e.g.: UK-14304 and B-HT 920) fail to elicit such a response but are sensitive to low concentrations of prazosin (< 0.1 μM).

(b) The two α-adrenoceptor subtypes reside on the same cells and interact at the level of a common post-receptor site in the events leading to contraction. Thus, responses mediated by postjunctional α_2-adrenoceptors on the rabbit isolated saphenous vein (the major subtype) may be facilitated by (or dependent upon) a small degree of stimulation of the α_1-adrenoceptor population: an interaction which would render the contractile response 'prazosin-sensitive'. Even though the action of prazosin at α_1-adrenoceptors would be competitive, this would not appear to be the case from the shift in the agonist concentration/tissue-response curve. Similarly the quantitative shifts produced by other antagonists might well fail to satisfy the criteria for competitive antagonism because of their unequal influence on the two sets of receptor-mediated events (e.g. rauwolscine and CH-38083).

An analogous situation appears to exist for β_1- and β_2-adrenoceptors that mediate relaxation to NA in guinea-pig isolated tracheal strips (Carswell & Nahorski, 1983). Here, the existence of the β_1-subtype was clearly evident from the presence of a 'resistant-component' to NA in the presence of the selective β_2-adrenoceptor antagonist ICI 118,551. However, with increasing concentrations of the selective β_1-adrenoceptor antagonist atenolol, the NA CRCs were displaced in a concentration-dependent parallel manner, with no evidence of a β_2-adrenoceptor-mediated resistant component, and the slope of the Schild plot was less than unity. The authors suggested that this was indicative of a functional interaction between two subtypes of adrenoceptor that mediate the same response. Similarly, Shepperson (1984) has demonstrated an even more subtle interaction between a functionally active population of α_1-adrenoceptors and a 'quiescent' population of α_2-adrenoceptors on the cat nictitating

membrane. Contractile responses to selective α_1-adrenoceptor agonists were potentiated by prior exposure to a selective α_2-adrenoceptor agonist even though these agonists failed to elicit a functional response, and this effect was inhibited by selective α_2-adrenoceptor antagonists. This type of interaction has also been proposed for α_1- (active) and α_2-adrenoceptors (quiescent) in the rabbit isolated femoral artery (Gisclard *et al.*, 1987).

Since the original demonstration of postjunctional α_2-adrenoceptors in the pithed rat and pithed cat (Drew & Whiting, 1979; Flavahan & McGrath, 1980), identification of this subtype on isolated blood vessels (that both respond to NA and are resistant to prazosin) has proved to be more difficult than for prejunctional α_2-adrenoceptors. Although numerous suggestions have been advanced to account for the elusive nature of this subtype (see: McGrath, 1982; Weiss *et al.*, 1983; Hieble *et al.*, 1986), it is clear from the results presented here that various factors may conspire to render contractions mediated by postjunctional α_2-adrenoceptors 'prazosin-sensitive', i.e.; in the blood vessels currently available for study, activation of α_1-adrenoceptors by the agonist may be necessary for (or may exert pronounced facilitatory effects upon) the α_2-adrenoceptor-mediated response.

In connection with this, several studies on the rat isolated tail artery have yielded conflicting results on the nature of the postjunctional α-adrenoceptor(s) mediating contractions to NA and other agonists (Weiss *et al.*, 1983; Medgett & Langer, 1984; Marwood *et al.*, 1986; Abel & Minneman, 1986; Rajanayagam & Medgett, 1987). While most investigators accept that an 'α_1-like'-adrenoceptor is the major subtype present, the possible contribution of functional postjunctional α_2-adrenoceptors is still questioned. However, a number of observations from these studies are essentially similar to those obtained for the rabbit isolated saphenous vein. First, low concentrations of a selective α_2-adrenoceptor antagonist, idazoxan, produced a preferential inhibition of the lower portion (25% of maximum) of a NA concentration-response curve constructed in the presence of prazosin (Rajanayagam & Medgett 1987). Secondly, Su *et al.* (1986) have shown that the selective α_2-adrenoceptor antagonists yohimbine and idazoxan produced an apparently non-competitive inhibition of contractile responses elicited by NA and by agonists selective for α_1- and α_2-adrenoceptors, with pA_2 values intermediate between anticipated values for interaction with either subtype. Although the authors explained this by invoking a single population of α-adrenoceptors in the rat tail artery different from both the α_1- and α_2-adrenoceptors described *in vivo*, this is not a satisfactory explanation for the rabbit isolated saphenous vein where other observations indicate the presence of two discrete receptors (results herein). Indeed, there may be many other examples where an α_2-adrenoceptor-mediated response has been missed through its indirect sensitivity to prazosin resulting from a functional interaction with α_1-adrenoceptors.

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