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An attempt at selective protection from phenoxybenzamine of postjunctional α-adrenoceptor subtypes mediating contractions to noradrenaline in the rabbit isolated saphenous vein

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1 An attempt has been made, with the irreversible α-adrenoceptor antagonist phenoxybenzamine, to find the conditions under which postjunctional α_1-adrenoceptors in the rabbit isolated saphenous vein can be inactivated, such that postjunctional α_2-adrenoceptors can be studied in isolation.

2 Following exposure to various concentrations of phenoxybenzamine, no evidence was found for a selective inactivation of the postjunctional population of α_1-adrenoceptors: the 'rauwolscine-resistant' (α_1-) and the 'rauwolscine-sensitive' (α_2-) responses to (−)-noradrenaline were similarly affected.

3 However, in 'receptor protection' experiments following exposure to a combination of phenoxybenzamine and the selective α_2-adrenoceptor antagonist rauwolscine, the remaining response to (−)-noradrenaline appeared to be mediated by a single population of postjunctional α_2-adrenoceptors: the response was insensitive to prazosin and rauwolscine was more potent than corynanthine.

4 Partial isolation of the α_1-adrenoceptor population was attempted by pre-exposure of the preparation to a combination of phenoxybenzamine and a selective α_1-adrenoceptor antagonist, i.e. prazosin or YM-12617. Following receptor protection, the inhibition produced by 'selective' concentrations of either of these α_1-adrenoceptor antagonists were not significantly different from that observed in control preparations (no phenoxybenzamine). However, the selective α_2-adrenoceptor antagonists rauwolscine and CH-38083 were still able to inhibit part of the remaining responses to NA. This is interpreted as indicating that, in addition to protecting the putative postjunctional α_1-adrenoceptors, these procedures fail to produce complete inactivation of postjunctional α_2-adrenoceptors.

5 It is concluded that, although phenoxybenzamine appeared to be non-selective for the two populations of postjunctional α-adrenoceptors in the rabbit isolated saphenous vein, inclusion of a 'selective' concentration of a competitive antagonist during the inactivation period results in differing degrees of functional protection of each subtype. Pharmacological isolation was possible for α_2-adrenoceptors but not convincingly for α_1-adrenoceptors.

Introduction

Based upon the effects of various selective α-adrenoceptor antagonists on contractile responses to (−)-noradrenaline (NA), we have suggested that the post-junctional α-adrenoceptor population of the rabbit isolated saphenous vein consists of a mixture of α_1- and α_2- subtypes (Daly *et al.*, 1988a,b). In other preparations with a mixture of both receptors (e.g. dog isolated saphenous vein—Flavahan *et al.*, 1984; Ruffolo & Zeid, 1985), the α_2- subtype has been studied in isolation by the use of selective non-phenethylamine agonists (e.g. azepexole and UK-14304) alone or in combination with a selective α_1-adrenoceptor antagonist. This particular approach, however, depends not only upon the (assumed) selectivity of both the agonists and antagonist for their respective subtypes but also upon the assumption that there is no 'break-through' agonism at the other receptor subtype. This problem invariably results in the exclusion of NA, the definitive α-adrenoceptor agonist (Furchgott 1972), from

further studies. In the rabbit isolated saphenous vein responses to both non-selective (NA) and selective α_2-adrenoceptor (UK-14304) agonists alike, are antagonized by the selective α_1-adrenoceptor antagonist prazosin (Cambridge *et al.*, 1977) in a non-competitive manner over a wide concentration range (Purdy *et al.*, 1980; Schümann & Lues 1983; Daly *et al.*, 1988b). Thus, the absence of a clear-cut prazosin-resistant component of responses to NA in this preparation not only precludes the possibility of examining the α_2-subtype in isolation, but represents a challenge to the view that the identification of postjunctional α_2-adrenoceptors is critically dependent upon the demonstration of such a component (see: Docherty & Starke, 1981; McGrath, 1982).

The irreversible α-adrenoceptor antagonist phenoxybenzamine (Furchgott, 1972) has been shown to exhibit selectivity for the α_1-subtype in a variety of isolated preparations from different species (Dubocovich & Langer, 1974; Doxey *et al.*, 1977; Borowski *et al.*, 1977), and to inhibit pre- and postjunctional α_2-adrenoceptors only at very high concentrations (Constantine *et al.*, 1982; Flavahan *et al.*, 1984; Hölting & Starke, 1986). We have, therefore, examined the effect of phenoxybenzamine on contractile responses to NA in the rabbit saphenous vein for two reasons. Firstly, to confirm our view that both α_1- and α_2-adrenoceptors exist in this preparation and, secondly, to establish conditions under which the α_2-adrenoceptor population can be examined in detail with NA, free of the influence of the α_1-adrenoceptor population.

Methods

Albino rabbits of either sex weighing between 2.2–3.3 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 length 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 isometric 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 g and allowed to relax. Each preparation was bathed in PSS maintained at 37°C and gassed with 95% O_2 plus 5% CO_2.

After a 60 min equilibration period, during which a steady resting tension of 0.4–0.6 g was achieved, each preparation was exposed to 3 μM 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 of Ruffolo *et al.* (1979). Basal tension following the sighting response (0.35–0.5 g) remained stable for the rest of the experiment. Isometric contractions were recorded by a Grass FT03 transducer connected to a Linseis 6025 pen recorder.

In all experiments, cumulative concentration-response curves (CRC) to NA were constructed by increasing the concentration of NA in the organ bath by approximately 3 fold increments following attainment of the peak response. In the majority of preparations contractile responses to NA were not sustained and addition of the next concentration was made as close to the peak as possible. Following attainment of the maximum control contraction, preparations were washed until complete relaxation was effected and one of three experimental protocols was adopted (see Figure 1).

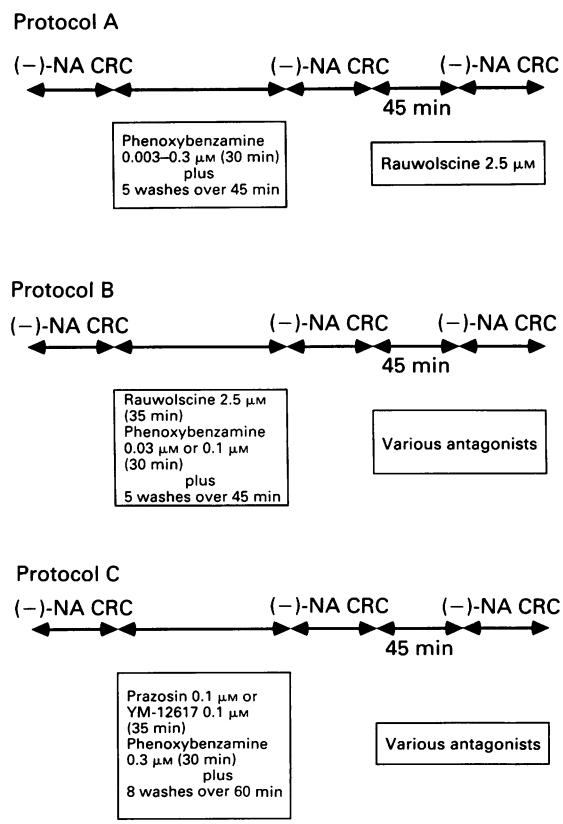

Figure 1 Schematic representation of the receptor inactivation protocols (A, B and C) employed. (−)-NA CRC = (−)-noradrenaline concentration-response curve.

Protocol A

Preparations were exposed to a single concentration of phenoxybenzamine (3 nM, 0.01 μM, 0.03 μM, 0.1 μM or 0.3 μM) for 30 min and then washed a minimum of five times over the next 45 min before the NA CRC was repeated. The preparations were again washed several times to effect complete relaxation and then exposed to 2.5 μM rauwolscine for a further 45 min. The NA CRC was then repeated.

Protocol B

Preparations were exposed to 2.5 μM rauwolscine 5 min before the addition of either 0.03 μM or 0.1 μM phenoxybenzamine and both antagonists were then washed out after a 30 min period (total time: 35 min for rauwolscine; 30 min for phenoxybenzamine). The preparations were washed a minimum of five times over a 45 min period and a concentration-response curve to NA was constructed. Twenty min after washout various antagonists were added and 45 min later the NA CRC was repeated.

Protocol C

Preparations were exposed to 0.1 μM prazosin or 0.1 μM YM-12617 5 min before the addition of 0.3 μM phenoxybenzamine and both antagonists were then washed out after a 30 min period (total time: 35 min for prazosin or YM-12617; 30 min for phenoxybenzamine). The preparations were then washed a minimum of eight times over a 45 min period and a concentration-response curve to NA was constructed. Twenty min after washout various antagonists were added and 45 min later the NA CRC was repeated.

All responses are represented as a percentage (mean $\pm$ s.e.mean) of the maximum response of the first CRC. Differences between means were considered significantly different if $P < 0.05$ for either paired or unpaired observations (Student's t test). For experiments performed under protocol B the logarithm of the agonist concentration-ratios produced by the antagonists were determined at the 50% level of the maximum response following receptor inactivation. For experiments performed under protocol C, a significant increase in the maximum response was observed between the two CRC constructed after receptor protection and, therefore, the logarithm of the agonist concentration-ratio was determined at the 50% level of the maximum of each individual CRC. Where possible, pA_2 values were determined by the method of Arunlakshana & Schild (1959).

The composition of the PSS was (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, NaH_2CO_3 24.9, KH_2PO_4 1.2 and glucose 11.1. Na_2 EDTA 0.023 mM was included in all experiments to prevent oxidative degradation of NA, and 1 μM propranolol and 10 μM cocaine were also included to inhibit β-adrenoceptors and uptake$_1$, respectively.

The following drugs were used: (−)-noradrenaline bitartrate (Sigma). prazosin HCl (Pfizer), corynanthine HCl (Roth), rauwolscine HCl (Roth), phenoxybenzamine HCl (SK&F); BDF-6143 (4-chloro-2-(2-imidazolin-2-ylamino), isoindoline hydrochloride, Biersdorf AF, F.R.G.); YM-12617 (5-(2-((2-(2-ethoxyphenoxy) ethyl) amino) propyl)-2-methoxybenzene-sulphonamide HCl, Yamanouchi, Tokyo, Japan); CH-38083 (7,8-(methylenedioxi)-14-α-hydroalloberbane HCl, Chinoin, Budapest); ($\pm$)-propranolol HCl (Sigma) and cocaine (HCl) (Macarthys). With the exception of phenoxybenzamine, all drugs were dissolved in distilled water and added to the organ baths in a volume of 0.3 ml or less. Stock solutions of phenoxybenzamine (1 mM) were prepared in 20% absolute alcohol in distilled water and a drop of 1 N HCl was added to remove turbidity. Further dilutions were made in distilled water.

Results

Protocol A

Phenoxybenzamine (0.01–0.3 μM) produced a concentration-related rightward shift of the NA CRC which was associated with a reduction in the maximum response (Figure 2a). Phenoxybenzamine 0.003 μM failed to affect responses while 0.3 μM phenoxybenzamine abolished all responses to NA up to 30 μM ($n = 5$). In control preparations not exposed to phenoxybenzamine, 2.5 μM rauwolscine produced a change in the slope of the NA CRC which was associated with the appearance of a 'resistant component' (Figure 2b); a response sensitive to the selective α_1-adrenoceptor antagonist prazosin (Daly *et al.*, 1988b). The 'rauwolscine-resistant' component of responses to NA were also reduced in a concentration-dependent manner by prior exposure to phenoxybenzamine (Figure 2b).

Protocol B

The rationale behind this procedure was to protect the population of postjunctional α_2-adrenoceptors from phenoxybenzamine with 2.5 μM rauwolscine, a concentration that spared α_1-adrenoceptor-mediated responses (see above and Figure 2b). Following the inclusion of 2.5 μM rauwolscine before and during the incubation period with either 0.03 μM or 0.1 μM phenoxybenzamine the maximum response to NA was significantly increased compared with phenoxyben-

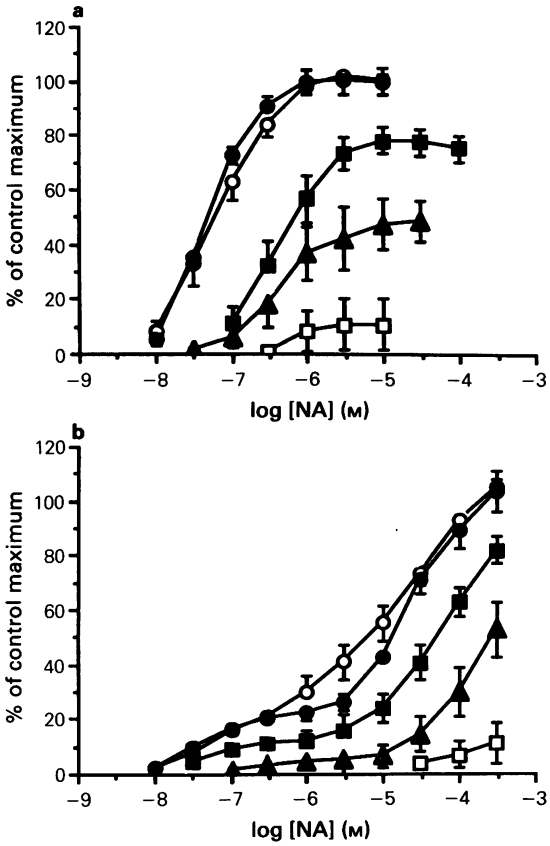

Figure 2 The effect of phenoxybenzamine on contractile responses to noradrenaline (NA) in the rabbit isolated saphenous vein. Preparations were exposed to either saline (○), 3 nM (●), 0.01 μM (■), 0.03 μM (▲) or 0.1 μM (□) phenoxybenzamine for 30 min and washed 5 times over 45 min (protocol A) and a CRC to NA constructed before (a) and 45 min after the inclusion of 2.5 μM rauwolscine (b) in the bathing medium. Responses were determined as a % of the maximum response to NA before phenoxybenzamine and are the mean of 5–9 observations with the vertical lines indicating the s.e.mean.

zamine alone ($P < 0.01$), from 48.7 ± 7.8% ($n = 6$) to 71.3 ± 3.2% ($n = 8$) or 10.7 ± 9.4% ($n = 6$) to 53.3 ± 3.5% ($n = 8$) of the control maximum, respectively. Coincident with this protective effect of 2.5 μM rauwolscine against 0.1 μM phenoxybenzamine, the 'rauwolscine-resistant' component of responses to NA was abolished (compare Figure 2b and 3d); rauwolscine now produced a concentration-dependent parallel displacement of the NA CRC (Figure 3d). The pA_2 value for rauwolscine was similar to that observed under control conditions but, in contrast to control, the slope was not significantly different from unity (Table 1b).

The log (agonist concentration-ratio) produced by 0.1 μM prazosin against NA following 2.5 μM rauwolscine and 0.03 μM phenoxybenzamine (0.38 ± 0.16, $n = 6$; Figure 3a) was significantly less than control (1.01 ± 0.04, $n = 7$), but was not significantly different from the value obtained following 2.5 μM rauwolscine and 0.1 μM phenoxybenzamine (0.19 ± 0.09, $n = 7$). Both 0.1 μM and 1 μM prazosin were significantly less effective against NA after 2.5 μM rauwolscine and 0.1 μM phenoxybenzamine compared with control preparations (Table 1a; Figure 3c). Figure 4 shows representative trace recordings of the effect of 0.1 μM prazosin on NA-induced contractions in normal preparations and those after exposure to both 2.5 μM rauwolscine and 0.1 μM phenoxybenzamine.

Protocol C

The rationale behind this procedure was to protect the population of postjunctional α_1-adrenoceptors from phenoxybenzamine with either 0.1 μM prazosin or 0.1 μM YM-12617. This concentration of prazosin has been employed to inhibit selectively α_1-adrenoceptors in the dog isolated saphenous vein (Flavahan *et al.*, 1984), while 0.1 μM YM-12617 is inactive at both prejunctional α_2-adrenoceptors in the rat isolated vas deferens (Honda *et al.*, 1985) and postjunctional α_2-adrenoceptors in the rabbit isolated ear vein (Daly *et al.*, 1988c).

Both 0.1 μM prazosin and 0.1 μM YM-12617 prevented the complete abolition of responses produced by 0.3 μM phenoxybenzamine. However, in approximately 25% of preparations, particularly those from young rabbits (<2.6 kg), the responses to NA were less than 10% of the original maximum, poorly maintained and subject to changes unrelated to the addition of NA to the bathing medium; these preparations were rejected from the subsequent quantitative analysis. The maximum responses in the remaining preparations varied from 10 to 35% of the control maximum response to NA. Unfortunately, successive CRC to NA following protection with either selective α_1-adrenoceptor antagonist were not reproducible; an approximate 30% increase in the maximum response occurred with no change in the sensitivity (based upon each individual CRC).

Following protection with 0.1 μM prazosin, approximately 50% of the response to NA was insensitive to 2.5 μM rauwolscine, while the upper component of the CRC was significantly shifted to the right with no change in the maximum response (Figure 5a). Prazosin 0.01 μM–1 μM produced a concentration-dependent inhibition of the NA CRC. This was associated with a significant increase in the maximum response in the presence of 0.01 μM and

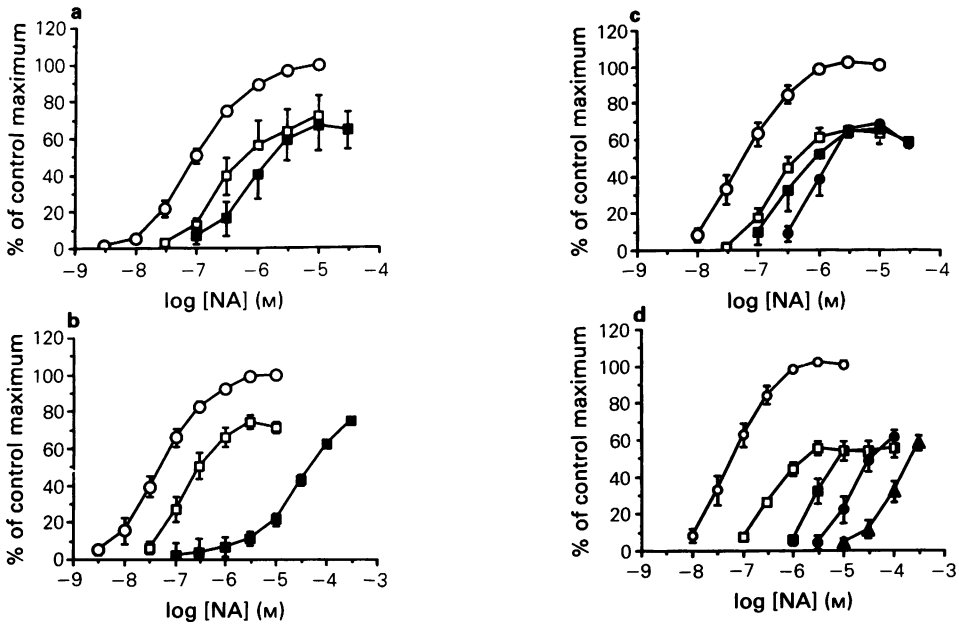

Figure 3 Concentration-response curves (CRC) to noradrenaline (NA) in the rabbit isolated saphenous vein before (○) and after partial inactivation of receptors and the effect, thereupon, of various antagonists. (a and b) Following exposure to a combination 0.03 μM phenoxybenzamine (30 min) and 2.5 μM rauwolscine (35 min) preparations were washed 5 times over 45 min (protocol B) and a NA CRC constructed before (□) and after either (a) 0.1 μM prazosin (■) or (b) 2.5 μM rauwolscine (■). (c and d) Following a combination of 0.1 μM phenoxybenzamine (30 min) and 2.5 μM rauwolscine (35 min) preparations were washed 5 times over 45 min (protocol B) and a NA CRC constructed before (□) and after either (c) 0.1 μM (■) and 1 μM prazosin (●) or (d) 0.05 μM (■), 0.5 μM (●) and 2.5 μM (▲) rauwolscine. Responses were determined as a % of the maximum response to NA before phenoxybenzamine and are the mean of 5–9 observations with the vertical lines indicating the s.e.mean.

0.1 μM prazosin, but not in the presence of 1 μM prazosin (Figure 5b). Based upon the slope of the Schild plot derived from the log (agonist concentration-ratio) at the 50% of the maximum for each CRC, prazosin effected a non-competitive inhibition (Table 1b).

Following receptor protection with 0.1 μM YM-12617, 2.5 μM rauwolscine produced a significant leftward displacement of the NA CRC (Figure 6b), while 0.1 μM YM-12617 (Figure 6a) effected a significant rightward displacement which, unlike 2.5 μM rauwolscine, was associated with an increase in the maximum response. The combination of 0.1 μM YM-12617 and 2.5 μM rauwolscine (Figure 6c) produced a greater inhibition than either antagonist alone and was not associated with a significant change in the maximum response. In marked contrast to 2.5 μM rauwolscine, the selective α_2-adrenoceptor antagonist CH-38083 (Vizi *et al.*, 1986), at a concentration of 1 μM, produced a significant rightward displacement of the NA CRC following exposure to 0.1 μM YM-12617 and 0.3 μM phenoxybenzamine to reveal a small 'resistant' component (Figure 6d).

The effect of other antagonists following receptor inactivation

Table 1 summarizes the results obtained with other α-adrenoceptor antagonists after 'selective' protection of one of the two subtypes with either 2.5 μM rauwolscine or 0.1 μM prazosin. Concentrations of BDF-6143 (15 nM) and corynanthine (2.5 μM) that effected similar shifts of the NA CRC in untreated preparations (approximately 10 fold; Daly *et al.*, 1988b) produced quantitatively similar inhibition of the two responses remaining after protocols B and C without changing the slope of the CRC. In contrast, the selective α_1-adrenoceptor antagonist YM-12617 (Honda *et al.*, 1985; Daly *et al.*, 1988c) produced a significantly greater inhibition of the response remaining after protocol C ('α_1-') than of the response remaining after protocol B ('α_2-').

Table 1 (a) Log agonist concentration-ratio values for (−)-noradrenaline (NA) following different antagonists under control conditions and after either control conditions, protocol B or C. (b) pA_2 values and slope of the Schild plot (with 95% confidence limits) for prazosin and rauwolscine under control conditions and after either protocol B or protocol C

Antagonist **a**	*Control*	*Protocol B*	*Protocol C*
Prazosin 0.1 μM	1.00 ± 0.04 $n = 7$	0.19 ± 0.09** $n = 7$	0.99 ± 0.09 $n = 9$
Prazosin 1 μM	1.43 ± 0.11 $n = 5$	0.64 ± 0.04** $n = 4$	1.51 ± 0.14 $n = 6$
Corynanthine 2.5 μM	0.77 ± 0.03 $n = 11$	0.82 ± 0.08 $n = 4$	0.59 ± 0.17 $n = 4$
BDF 6143 15 nM	0.92 ± 0.08 $n = 5$	1.30 ± 0.24 $n = 4$	1.25 ± 0.21 $n = 4$
YM-12617 0.1 μM	0.75 ± 0.08 $n = 8$	0.37 ± 0.08* $n = 6$	1.02 ± 0.1 $n = 4$
b	*Control*	*Protocol B*	*Protocol C*
Prazosin	8.44 (8.72–8.18) 0.58 (0.51–0.68) $n = 56$ individual points 0.005 μM–3 μM	<7 (see above)	8.71 (9.35–8.07) 0.58 (0.37–0.71) $n = 22$ individual points 0.01 μM–1 μM
Rauwolscine	8.56 (8.89–8.22)*** 0.85 (0.74–0.96) $n = 23$ individual points 0.05 μM–2.5 μM	8.33 (8.71–7.94) 0.87 (0.73–1.02) $n = 19$ individual points 0.05 μM–2.5 μM	Not possible (responses partially resistant)

Protocol B—NA CRC after prior exposure to 2.5 μM rauwolscine and 0.1 μM phenoxybenzamine. Protocol C—NA CRC after prior exposure to 0.1 μM prazosin and 0.3 μM phenoxybenzamine.
In (a) *significantly less than control $P < 0.01$; **significantly less than control $P < 0.001$.
In (b) with the exception of rauwolscine under control conditions (determined at the 75% level***) all log (agonist concentration-ratios) were determined at the 50% level of the maximum response.

Discussion

The irreversible α-adrenoceptor antagonist phenoxybenzamine produced a concentration-dependent rightward displacement of the NA CRC in the rabbit isolated saphenous vein and this was associated with a progressive reduction in the maximum response. The highest concentration employed (0.3 μM) abolished responses to NA. Qualitatively similar observations have been obtained in this preparation by both Schümann & Lues (1983) and Laher *et al.* (1986). In view of the number of studies suggesting that phenoxybenzamine possesses low affinity (in the μM range) for α_2-adrenoceptors (Dubocovich & Langer 1974; Doxey *et al.*, 1977; Hölting & Starke, 1986), the present observations are apparently at odds with the view that the rabbit isolated saphenous vein contains a population of postjunctional α_2-adrenoceptors (Schümann & Lues, 1983; Alabaster *et al.*, 1985; Daly *et al.*, 1988a,b).

Moreover, the 'rauwolscine-resistant, prazosin-sensitive' response to NA in this preparation, which was the basis for the further suggestion that both α_1- and α_2- subtypes are present (Daly *et al.*, 1988a,b), was not preferentially inhibited by phenoxybenzamine at any concentration (protocol A). Once again, this is inconsistent with the repeated observation in the dog isolated saphenous vein that low concentrations of phenoxybenzamine antagonize postjunctional α_1-adrenoceptors while leaving the postjunctional α_2-adrenoceptor population intact (Constantine *et al.*, 1982; Flavahan *et al.*, 1984; Ruffolo & Zeid, 1985). Nonetheless, the available evidence with the competitive antagonists rauwolscine and corynanthine indicates that the α-adrenoceptors in this preparation possess characteristics common to all other α_2-adrenoceptors; i.e.: rauwolscine > corynanthine (Daly *et al.*, 1988a).

The basis of the receptor protection experiments, undertaken with phenoxybenzamine, was to provide conditions whereby any postjunctional α_2- adrenoceptors (protocol B) or postjunctional α_1-adrenoceptors (protocol C) could be selectively protected by the inclusion of 'selective' concentrations of an antagonist. As evidenced by (1) the smaller reduction in the maximum response to NA

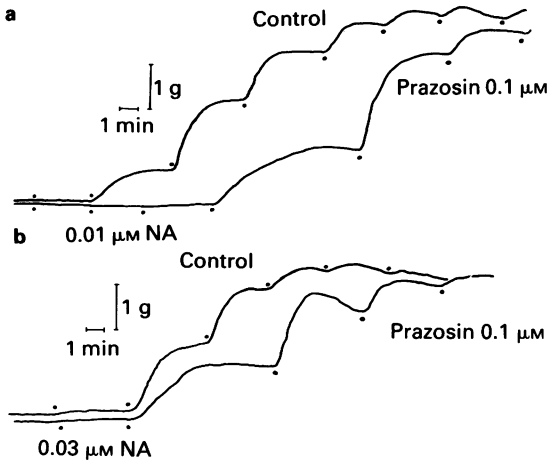

Figure 4 Representative trace recording of the effect of 0.1 μM prazosin on noradrenaline (NA)-induced contractions (cumulative addition; approximately 3 fold increments in concentration) of the rabbit isolated saphenous vein (a) in untreated preparations and (b) preparations following 30 min exposure to 2.5 μM rauwolscine and 0.1 μM phenoxybenzamine and subsequent washout (protocol B). In this particular experiment, preparations exposed to 0.1 μM phenoxybenzamine alone failed to respond to 10 μM NA (not shown).

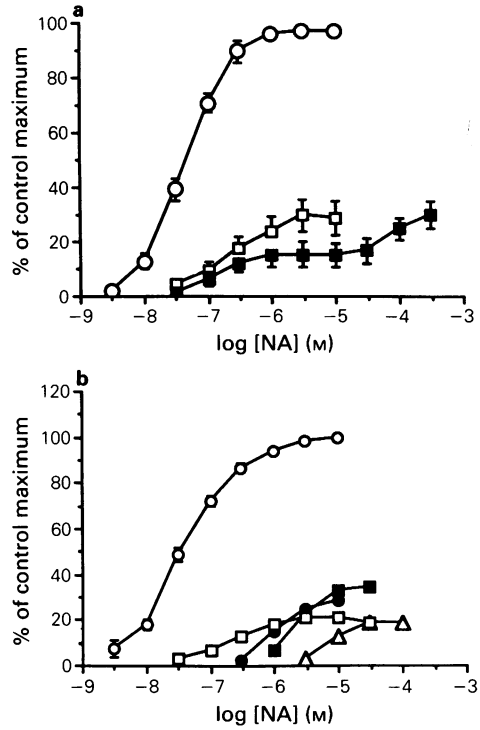

Figure 5 Concentration-response curves (CRC) to noradrenaline (NA) in the rabbit isolated saphenous vein before (○) and after partial inactivation of receptors and the effect, thereupon, of various antagonists. Following a combination of 0.3 μM phenoxybenzamine (30 min) and 0.1 μM prazosin (35 min) preparations were washed 8 times over 45 min (protocol C) and a NA CRC constructed before (□) and after either (a) 2.5 μM rauwolscine (■) or (b) 0.01 μM (●), 0.1 μM (■) and 1 μM (△) prazosin. Responses were determined as a % of the maximum response to NA before phenoxybenzamine and are the mean of 6–7 observations with the vertical lines indicating the s.e.mean. For the sake of clarity s.e.mean were omitted from (b) but for each CRC the values were less than (±6%).

following the combination of phenoxybenzamine with either rauwolscine, prazosin or YM-12617 compared to phenoxybenzamine alone, and (2) the different profile for the effects of the antagonists employed against the residual response, both procedures afforded some protection. It should be noted, however, that great care is needed in interpreting which receptor subtype is functionally active under the various conditions.

After protocol B, contractions to NA were antagonized by rauwolscine in a competitive manner with no evidence of a 'resistant' component, while both prazosin (0.1 μM–1 μM) and YM-12617 (0.1 μM) were significantly less effective against these responses than against responses under either control conditions or after protocol C. This is consistent with a discrete population of postjunctional α_2-adrenoceptors which are preferentially protected by rauwolscine. The low potency of prazosin at this isolated subtype ($pA_2 < 7$) agrees well with that found by Schümann & Lues (1983) in this preparation against B-HT 920-induced contractions in the presence of angiotensin II ($pA_2 = 6.8$). Furthermore, the low potency of YM-12617 is also consistent with an action at α_2-adrenoceptors (Honda *et al.*, 1985).

In contrast to the relatively clear-cut observations obtained after receptor protection with rauwolscine, protection of the 'α_1-like' adrenoceptors with either 0.1 μM prazosin or 0.1 μM YM-12617 did not yield straightforward results (protocol C).

First, approximately 50% of the residual response following protection with prazosin was sensitive to 2.5 μM rauwolscine, while prazosin behaved as a non-competitive antagonist against the remaining response to NA with a pA_2 value similar to that observed under control conditions (Table 1b). If only α_1-adrenoceptors had remained then prazosin might have been expected to be a competitive antagonist. Corynanthine (2.5 μM), however, caused a 4 fold parallel displacement of the NA CRC (Table 1a) and, thus, for part of the response at least, was more

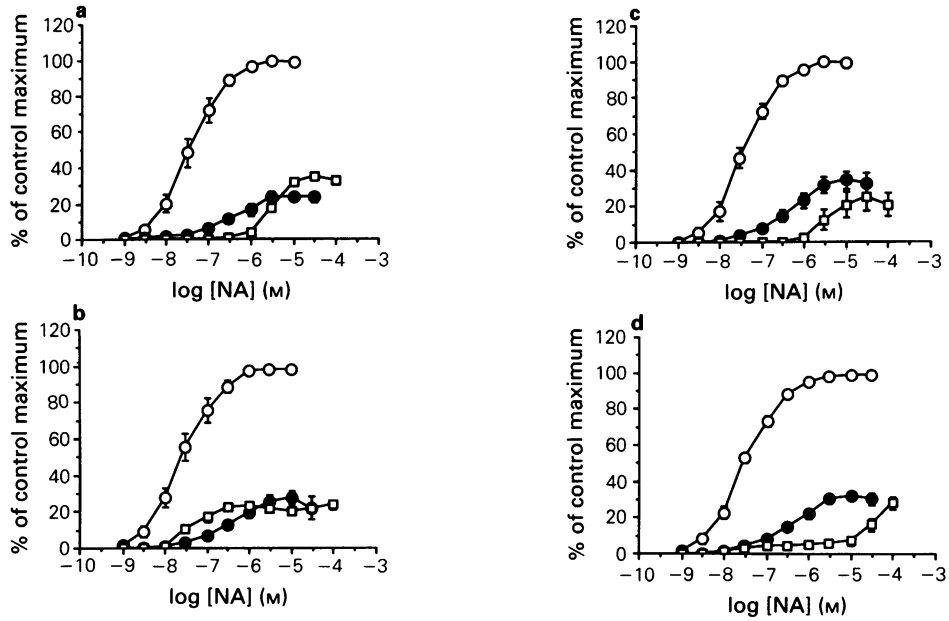

Figure 6 Concentration-response curves (CRC) to noradrenaline (NA) in the rabbit isolated saphenous vein before (○) and after partial inactivation of receptors and the effect, thereupon, of various antagonists. Following a combination of 0.3 μM phenoxybenzamine (30 min) and 0.1 μM YM-12617 (35 min) preparations were washed 8 times over 45 min (protocol C) and a NA CRC constructed before (●) and after either (a) 0.1 μM YM-12617 (□), (b) 2.5 μM rauwolscine (□), (c) a combination of 0.1 μM YM-12617 and 2.5 μM rauwolscine (□) and (d) 1 μM CH-38083 (□). Responses were determined as a % of the maximum response to NA before phenoxybenzamine and are the mean of 5–7 observations with the vertical lines indicating the s.e.mean.

potent than its diastereoisomer rauwolscine. Therefore, a sizeable component of the response to NA appears to possess the characteristics of an α_1-subtype (corynanthine > rauwolscine: see Daly *et al.*, 1988a).

Since the concentration of phenoxybenzamine employed (0.3 μM) in protocol C abolished responses to NA in the absence of protection, the responses blocked by rauwolscine after protocol C could be mediated by receptors protected by prazosin. This might arise if, in addition to protecting postjunctional α_1-adrenoceptors, limited protection of the α_2-subtype is afforded by the inclusion of 0.1 μM prazosin during the incubation period. While there is always the problem of circularity in this assessment of the results, the selectivity of 2.5 μM rauwolscine for postjunctional α_2-adrenoceptors was based upon the finding that in this preparation, under control conditions, the 'rauwolscine-resistant' component to NA (prazosin-sensitive; α_1-) was most pronounced with this concentration (see; Figure 2d Daly *et al.*, 1988b). Alternatively a residual α_2-component which survives phenoxybenzamine, but cannot reach threshold for contraction on its own, may facilitate the response mediated by the α_1-adrenoceptors protected by prazosin and YM-12617.

Secondly, following protection with 0.1 μM YM-12617, rauwolscine increased the sensitivity of the preparation to NA (Figure 6b), but 0.1 μM YM-12617 itself was no more effective than in preparations not exposed to phenoxybenzamine (Table 1a). For YM-12617, this represents a considerably lower potency than observed at α_1-adrenoceptors in the rabbit aorta (Honda *et al.*, 1985) or renal vein (unpublished observations); pA_2 approximately 9.5.

Thirdly, a significant increase in the maximum response to NA was observed between successive CRC (in the absence of an antagonist) following protection with 0.1 μM YM-12617. This was also noted when the second CRC was repeated in the presence of 0.1 μM YM-12617 (Figure 6a). The combination of 2.5 μM rauwolscine and 0.1 μM YM-12617 (Figure 6c) prevented the time-dependent increase in the maximum response, but failed to produce a further, rightward displacement of the NA CRC, as might have been anticipated from a combination of selective antagonists in a two receptor system (McGrath, 1982).

Finally, the selective α_2-adrenoceptor antagonist CH-38083 (Vizi *et al.*, 1986), which also revealed a 'resistant' component to NA in untreated preparations of the saphenous vein (Daly *et al.*, 1988b), effected a marked inhibition of responses following protection with 0.1 μM YM-12617 and uncovered a 'resistant' component considerably smaller than that seen with 2.5 μM rauwolscine (compare b and d in Figure 6). Thus, after protection by YM-12617, responses are resistant to one α_2-adrenoceptor antagonist, rauwolscine, but susceptible to another, CH-38083, underlining the complex effects of prazosin protection (see above). This suggests that the protected contractile response to NA following protocol C is mediated by a heterogeneous population of α-adrenoceptors, and emphasizes further the failure of the procedure to isolate the α_1- subtype.

Overall these observations represent something of a paradox. Both 0.1 μM prazosin and 0.1 μM YM-12617 are considered to be selective for α_1-adrenoceptors in the dog isolated saphenous vein (Flavahan *et al.*, 1984; Honda *et al.*, 1985), and we have also demonstrated that they are relatively inactive at postjunctional α_2-adrenoceptors in the rabbit isolated ear vein (Daly *et al.*, 1988c). However, these concentrations of the antagonists were capable of protecting a population of post-junctional α-adrenoceptors from inactivation by phenoxybenzamine, presumably by occupying the same site as phenoxybenzamine, but then failed to effect appreciable inhibition of the remaining responses elicited by NA.

It has been suggested that the diversity of chemical structures capable of inhibiting α-adrenoceptors can be taken as evidence that some antagonists produce their effects by attachment to 'extra-receptors' sites not necessarily corresponding to the area of agonist attachment (see: McGrath, 1982; Ariens & Simonis, 1983). If this is also correct for postjunctional α_2-adrenoceptors in the rabbit isolated saphenous vein, it seems possible that both prazosin and YM-12617 may compete with phenoxybenzamine at a critical 'extra-receptor' site, thereby preventing irreversibile inactivation but without hindering the access of the agonist to the receptor. Thus, following protocol C contractile responses to NA would result from the co-stimulation of a population of postjunctional α_1-adrenoceptors and a population of post-junctional α_2-adrenoceptors. The presence of two postjunctional α-adrenoceptor subtypes that interact synergistically (Daly *et al.*, 1988b) would, of course, preclude meaningful quantitative assessment of the actions of selective antagonists. While this may account for the subsequent resistance to prazosin and YM-12617 of responses to NA, it does not explain the potentiation of responses observed with rauwolscine (Figure 6b).

Concentrations of the antagonists BDF-6143 and corynanthine that produced approximately 10 fold parallel shift of the NA CRC under control conditions (Table 1), effected similar inhibition of the responses following the incubation with phenoxybenzamine and protection with either prazosin or rauwolscine. This accords with the competitive inhibition produced by both of these antagonists against NA contractions under control conditions (Daly *et al.*, 1988b) and suggests that the supposed selectivity of these compounds for prejunctional α_2-adrenoceptors in the rabbit main pulmonary artery (BDF 6143; Docherty *et al.*, 1982) and post-junctional α_1-adrenoceptors in the rat isolated vas deferens and rat isolated anococcygeus (corynanthine; McGrath, 1982) is not evident in this preparation. However, it may be relevant that neither antagonist has been tested previously against postjunctional α_2-adrenoceptors *in vitro*. Clearly, we need not be bound by 'rules' established by antagonist potency series on prejunctional α_2-adrenoceptors (see also below on SK & F 104078). Further caution is warranted in the interpretation of blockade following protocol C where more than one receptor may be involved and, where antagonist-induced changes in the response were particularly difficult to assess because of time-dependent increases in the magnitude of the response (in the absence of antagonists): these often exceeded 30% of the post-phenoxybenzamine response. Interestingly, while these changes were particularly pronounced in the presence of either low concentrations of prazosin or 0.1 μM YM-12617, they were not observed when rauwolscine or CH-38083 was employed, thus indicating further that α_2-adrenoceptors make a significant contribution to contractions elicited by NA following protocol C.

The results from this study appear to support our previous suggestion that in untreated preparations of the rabbit isolated saphenous vein, a small population of 'α_1-like' adrenoceptors exerts a pronounced effect on the major subtype present, the α_2-adrenoceptor population (Daly *et al.*, 1988b). This renders contractions to NA and 'selective' α_2-adrenoceptor agonists sensitive to low concentrations of prazosin. Coincidentally, with the elimination of the 'rauwolscine-resistant' response to NA following protocol B, the preparations were 1/10th as sensitive to NA and the contractions were resistant to 0.1 μM prazosin. Two examples of a functional synergism between agonists/receptors have recently been demonstrated in guinea-pig liver cells (Cocks *et al.*, 1984) and in rat pinealocytes (Sugden *et al.*, 1984; Vanecek *et al.*, 1985). Interestingly, in both instances the synergism was attributed to an interaction at the level of the secondary messenger system employed by each receptor. Since, α_1-adrenoceptors appear to

be coupled with the inositol phosphate signalling system while α_2-adrenoceptors may be linked to changes in adenylate cyclase activity (Exton, 1985), it may be speculated that a similar interaction accounts for the observations in the present study.

Phenoxybenzamine (0.003 μM–0.1 μM) failed to effect a selective inhibition of postjunctional α_1-adrenoceptors, as evidenced by the need to include rauwolscine to protect the α_2-adrenoceptor population (protocol B). This suggests that postjunctional α_2-adrenoceptors on the rabbit isolated saphenous vein may differ from the postjunctional population present in the dog isolated saphenous vein (Constantine *et al.*, 1982; Flavahan *et al.*, 1984) and those located on prejunctional sites on the rabbit isolated pulmonary artery (Borowski *et al.*, 1977) and rabbit cerebral cortex (Holting & Starke, 1986). This latter study is of particular interest since it was shown that 1 μM prazosin failed to prevent irreversible inactivation of prejunctional α_2-adrenoceptors by 0.1 μM phenoxybenzamine. Further evidence in favour of intra-species differences between pre- and post-junctional α_2-adrenoceptors has recently been suggested by the observation that the α-adrenoceptor antagonist SK&F 104078 possesses 100 fold greater affinity for postjunctional sites (Hieble *et al.*, 1986) and by the high potency of corynanthine at postjunctional α_2-adrenoceptors in the rabbit isolated ear vein (see Daly *et al.*, 1988a).

In conclusion, based upon the receptor protection experiments in the rabbit isolated saphenous vein with 'selective' concentrations of either α_1- or α_2-adrenoceptor antagonists in combination with the irreversible antagonist phenoxybenzamine, two populations of postjunctional α-adrenoceptors can be identified. One population has the characteristics of an α_2-adrenoceptor (prazosin-resistant, rauwolscine > corynanthine), while the other possesses some of the characteristics of an α_1-adrenoceptor (prazosin-sensitive, rauwolscine-resistant). Although complete isolation of the α_2-subtype could be achieved, only limited success was attained in isolating the 'α_1-' subtype. Thus, the physiological and pharmacological characteristics of the postjunctional α_2-adrenoceptors can be examined with the 'endogenous' agonist NA without the need to include either a selective α_1-adrenoceptor antagonist in the bathing medium (e.g. Flavahan *et al.*, 1984) or various other ancillary drugs (Schümann & Lues, 1983; Sulpizio & Hieble, 1987).

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