Postjunctional α -adrenoceptors in the rabbit isolated distal saphenous artery: indirect sensitivity to prazosin of responses to noradrenaline mediated via postjunctional α_2 -adrenoceptors

¹W.R. Dunn, J.C. McGrath & ²V.G. Wilson

Autonomic Physiology Unit, Institute of Physiology, University of Glasgow, Glasgow, G12 8QQ

1 Under normal experimental conditions, the rabbit isolated distal saphenous artery appears to contain a homogeneous population of postjunctional α_1 -adrenoceptors. Prazosin competitively antagonized responses to noradrenaline (NA) with a pA_2 value of 8.6, while a relatively high concentration of rauwolscine ($1 \mu M$), produced only a 2 fold rightward displacement of the NA cumulative concentration-response curve (CCRC).

2 Despite the fact that angiotensin II (AII) was without effect on responses to NA or phenylephrine in this preparation, this peptide made responses to NA less susceptible to the antagonistic action of prazosin. This was particularly evident on the lower portion of the CCRC for NA. These results suggest that in the presence of AII, NA produces contractile responses by an action mediated through a prazosin-resistant adrenoceptor.

3 An attempt was made to isolate a homogeneous population of postjunctional α_2 -adrenoceptors by use of a receptor protection procedure involving the combination of rauwolscine and phenoxybenzamine. After the protection protocol no responses were observed to the α -adrenoceptor agonists NA, phenylephrine or UK-14304. In the presence of angiotensin II however, concentration-dependent contractions were observed to each of these agonists. Under these conditions the rank order of potency, UK-14304 > NA > phenylephrine, is consistent with that of an effect mediated through postjunctional α_2 -adrenoceptors.

4 The responses to NA, after the protection protocol, in the presence of AII, were susceptible to the selective α_2 -adrenoceptor antagonist, rauwolscine ($1 \mu M$), but resistant to the selective α_1 -adrenoceptor antagonist prazosin ($0.1 \mu M$). Furthermore, the combination of rauwolscine ($1 \mu M$) and prazosin ($0.1 \mu M$) was no more effective in blocking responses to NA than was rauwolscine ($1 \mu M$) alone. These results are consistent with the presence of a homogeneous population of postjunctional α_2 -adrenoceptors.

5 Inducing a small degree of tone with a low concentration of the selective α_1 -adrenoceptor agonist, phenylephrine, markedly increased the threshold sensitivity to the selective α_2 -adrenoceptor agonist UK-14304, in a manner analogous to that seen with AII.

6 The results in the present study indicate that responses mediated via postjunctional α_2 -adrenoceptors in the rabbit isolated distal saphenous artery are dependent upon a degree of vascular smooth muscle stimulation by some other receptor system. It is hypothesized that under normal experimental conditions, this function is fulfilled by stimulation of α_1 -adrenoceptors, while after α_1 -adrenoceptor blockade the necessary positive influence can be provided by stimulation of AII receptors. The implications for such an interaction between postjunctional α -adrenoceptor subtypes in demonstrating prazosin-resistant, rauwolscine- or yohimbine-sensitive responses in isolated blood vessels is discussed.

Keywords: Noradrenaline; angiotensin II; postjunctional α_1 -adrenoceptors; postjunctional α_2 -adrenoceptors; rabbit isolated distal saphenous artery; interaction

Introduction

In contrast to the relative ease of demonstrating postjunctional α_2 -adrenoceptor-mediated pressor responses in whole animals, it has been much more difficult to show prazosin-resistant, rauwolscine- or yohimbine-sensitive responses in isolated vascular preparations (McGrath *et al.*, 1989). Some success has been achieved in several isolated venous preparations such as the canine saphenous vein (Constantine *et al.*, 1982), rat saphenous vein (Cheung, 1985), rabbit saphenous vein (Daly *et al.*, 1988a) and most convinc-

ingly, the rabbit ear vein (Daly *et al.*, 1988b). It has been even more difficult however, to demonstrate unequivocally, responses mediated via postjunctional α_2 -adrenoceptors in isolated arterial preparations, the likely source of α_2 -adrenoceptor-mediated pressor responses *in vivo*.

Several explanations have been put forward to explain the elusive nature of this subtype *in vitro*. For example, it has been suggested that postjunctional α_2 -adrenoceptors are located on small arterioles which, because of their size, are not normally accessible for examination *in vitro* (McGrath, 1982). This hypothesis is supported by the recent observations that functional postjunctional α_2 -adrenoceptors are present on rat cremaster arterioles (Faber, 1988) and on human resistance vessels, where an inverse relationship between vessel diameter and magnitude of α_2 -adrenoceptor-mediated responses was observed (Neilsen *et al.*, 1989). An additional explanation for the difficulty in demonstrating postjunctional α_2 -adrenoceptors *in vitro* may be the lack of humoral agents

¹ Present address: Department of Pharmacology, University of Vermont, Given Medical Building, Burlington, Vermont, 05405, U.S.A.

² Author for correspondence at present address: Department of Physiology and Pharmacology, Medical School, Queens Medical Centre, Clifton Boulevard, Nottingham, NG7 2UH.

normally present in the whole animal (Schümann & Lues, 1983). For example it has been reported that the expression of postjunctional α_2 -adrenoceptor-mediated responses to UK-14304 in the rabbit distal saphenous artery is dependent upon the presence of angiotensin II (AII) (Dunn *et al.*, 1989). Similarly, in the perfused isolated tail of the rat α_2 -adrenoceptor-mediated responses are observed only after raising tone with, for example vasopressin or endothelin (Templeton *et al.*, 1989; MacLean & McGrath, 1990), while in the canine saphenous artery a non-contractile concentration of Bay K 8644 allowed the demonstration of responses mediated by the subtype (Sulpizio & Hieble, 1987).

The above mentioned studies with vasoactive agents have demonstrated the expression of α_2 -adrenoceptor-mediated responses to synthetic agonists which are relatively selective for α_2 -adrenoceptors. The present study has addressed the influence of AII on responses to the endogenous ligand NA in the rabbit isolated distal saphenous artery. The results demonstrate that functional postjunctional α_2 -adrenoceptors, responding to NA, are present in this preparation, despite the fact that under 'standard' experimental conditions responses to NA are sensitive to low concentrations of the selective α_1 -adrenoceptor antagonist, prazosin. Our observations suggest an interaction between postjunctional α_1 - and α_2 -adrenoceptors, which may be of more general applicability for other isolated vascular preparations.

Methods

White albino rabbits of either sex weighing 2.3–3.0 kg were killed by stunning followed by exsanguination. Segments of the distal saphenous artery were cleaned of fat and connective tissue *in situ* and placed in ice-cold physiological solution (PSS). When necessary, preparations were cleaned further with the aid of a dissecting microscope. Subsequently, 3–5 mm length segments were taken from each preparation and suspended between two 0.2 mm thick 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. Each segment was then mounted in 30 ml isolated organ baths under an initial resting tension of 2 g wt. and allowed to relax. Each preparation was bathed in PSS maintained at 37°C and gassed with 95% O₂ plus 5% CO₂.

After a 60 min equilibration period, during which a steady resting tension was achieved, each preparation was exposed to 3 μ M NA and allowed to contract for 10 min. Following complete washout, an additional equilibration period of 1 h was allowed before starting 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 remained stable for the rest of the experiment of 0.6–0.75 g wt. Isometric contractions were recorded by a Grass FT03 transducer connected to a Linseis 6025 pen recorder. In all experiments cumulative concentration-response curves (CCRCs) to agonists were constructed by increasing the concentration of agonist in the organ bath by approximately 3 fold increments. Following attainment of the maximal control contraction, preparations were washed until complete relaxation was effected and 30–60 min later one of the following experimental protocols adopted:

- (1) Preparations were exposed to AII (0.05 μ M) 15 min before the start of a second agonist CCRC.
- (2) Preparations were exposed to various concentrations of prazosin for a minimum period of 45 min and the CCRC for NA repeated. In some tissues, AII (0.05 μ M) was added to the organ bath 15 min before the start of the second CCRC to NA, in the presence of prazosin.
- (3) An attempt was made to isolate a population of postjunctional α_2 -adrenoceptors by use of a protocol similar to that

used by Daly *et al.* (1988a). Briefly, tissues were exposed to rauwolscine (1 μ M) 5 min prior to phenoxybenzamine (0.3 μ M) which was left in contact with the tissues for a further 60 min. The tissues were then washed a minimum of seven times over a 45 min period. During the first two washes rauwolscine (1 μ M) was present to maximize α_2 -adrenoceptor protection. After a further 60 min period a CCRC was obtained to various agonists. In some experiments AII was added 15 min prior to the onset of a further CCRC. In others, antagonists were applied at least 45 min before the start of an agonist CCRC.

(4) A low concentration of the selective α_1 -adrenoceptor agonist, phenylephrine, was applied to the distal saphenous artery to induce a small degree of tone. After a maintained contraction was achieved, a CCRC to the selective α_2 -adrenoceptor agonist UK-14304 was obtained.

All responses are expressed as a percentage (mean $\pm$ s.e.mean) of the maximum response of the first concentration-response curve to NA (or UK-14304) obtained in each tissue. Differences between means were considered statistically different if $P < 0.05$ for either paired or unpaired observations (Student's *t* test). In examining the effects of antagonists, agonist concentration-ratio values were determined from the concentrations producing 50% of the maximum response in the absence and presence of each concentration of antagonist. 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). According to Arunlakshana & Schild (1959) if antagonism is competitive, a plot of the logarithm of dose-ratio – 1 against the negative logarithm of the molar concentration of the antagonists, yields a straight line with a slope of one and the intercept of which along the abscissa scale is the pA_2 , which is equal to the antagonist dissociation constant (K_B) under equilibrium conditions. Antagonism was considered competitive if the 95% confidence limits for the slope of the Schild plot, drawn by linear regression, overlapped unity. In some experiments, the agonist concentration-ratio values in the presence of antagonist were determined at the 25%, 50% and 75% level of the maximum response to NA as described by Flavahan & Vanhoutte (1986).

The composition of the PSS was (mM): NaCl 118.4, KCl 4.7, CaCl₂ 2.5, MgSO₄·7H₂O 1.2, NaHCO₃ 24.9, KH₂PO₄ 1.2 and glucose 11.1. Na₂EDTA 23 μ M was included in all experiments to prevent oxidative degradation of NA and 1 μ M propranolol and 10 μ M cocaine were also included to inhibit β -adrenoceptors and uptake₁, respectively. The following drugs were used: (–)-noradrenaline bitartrate (Sigma), phenoxybenzamine HCl (Smith, Kline & French), rauwolscine HCl (Roth), angiotensin II amide (Ciba), (±)-propranolol HCl (Sigma), cocaine HCl (Macarthy), (–)-phenylephrine HCl (Sigma), UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate) (Pfizer) and prazosin HCl (Pfizer). With the exception of phenoxybenzamine, all drugs were dissolved in distilled water and added to the organ bath in volumes of 0.3 ml or less. Stock solutions of phenoxybenzamine (1 mM) were prepared in 20% absolute ethanol in distilled water and a drop of 1 N HCl was added to remove turbidity. Further dilutions were made in distilled water.

Results

Effects of antagonists on noradrenaline-induced contractions

As shown in Figure 1, prazosin produced a concentration-dependent, parallel rightward displacement of the CCRC to NA. The pA_2 value for prazosin was 8.57 and the 95% con-

Figure 1 Effects of the α -adrenoceptor antagonists (a) prazosin 0.01 μM (●), 0.1 μM (□) and 1 μM (■) and (b) rauwolscine (1 μM) (●) on responses to noradrenaline (NA, ○) in the rabbit isolated distal saphenous artery. Results are expressed as a % of the initial maximum response to NA. Each point represents the mean of $n = 5-6$; s.e.mean shown by vertical bars.

fidence interval for the slope of the Schild was not significantly different from unity, indicating competitive antagonism. Furthermore, prazosin (0.1 μM) produced an approximately 25 fold rightward displacement in the CCRCs to the α -adrenoceptor agonists, phenylephrine, Sgd 101/75 and UK-14304, an effect similar to that observed against NA. In contrast, a relatively high concentration of the selective α_2 -adrenoceptor antagonist rauwolscine (1 μM), produced only a 2 fold rightward shift in the NA CCRC (Figure 1b).

Effects of angiotensin II on responses to noradrenaline and phenylephrine

AII (0.05 μM) produced a transient contraction, which returned to baseline after 12–15 min, in the rabbit isolated distal saphenous artery. The peak response to AII was equivalent to $37.8 \pm 4.8\%$ ($n = 13$) of the maximum response to NA in each preparation. This agent had no effect on responses to the agonists NA (non-selective α_1/α_2) (Figure 2a) phenylephrine (selective α_1) (Figure 2b) or Sgd 101/75.

AII (0.05 μM) did, however, have a marked effect on the ability of prazosin to antagonize responses to NA in the distal saphenous artery (Figure 3). This was particularly marked for the lower portion of the CCRC to NA (cf. Figure 1a), so that the log agonist concentration-ratio value at the level of 25% of the maximum response to NA, in the presence of prazosin (0.1 μM), was significantly reduced from control values in the absence of AII (Table 1).

Figure 2 Effects of angiotensin II (0.05 μM) (●) on responses to the α -adrenoceptor agonists (a) noradrenaline (NA) and (b) phenylephrine (PE) in the rabbit isolated distal saphenous artery. Results are expressed as a % of the initial maximum response to each agonist (○). Each point represents the mean of $n = 5-6$; s.e.mean shown by vertical bars.

Attempted isolation of postjunctional α_2 -adrenoceptors

Phenoxybenzamine (0.3 μM) alone virtually abolished responses to NA in this preparation. The rationale employed therefore was to prevent phenoxybenzamine from binding to

Figure 3 The effects of 0.01 μM (●), 0.1 μM (□), and 1 μM (■) prazosin on responses of the rabbit isolated saphenous artery to noradrenaline (NA, ○) in the presence of 0.05 μM angiotensin II. Results are expressed as a % of the initial maximum response to NA. Each point represents the mean of $n = 5-6$; s.e.mean shown by vertical bars. c.f. Figure 1a.

Table 1 Log agonist concentration-ratio values for noradrenaline (NA) in the presence of prazosin (0.1 μ M) alone or in combination with angiotensin II (AII, 0.05 μ M) calculated at the levels of 25%, 50% and 75% of the maximum response to NA

Antagonist	25%	50%	75%
Prazosin (0.1 μ M) (<i>n</i> = 6)	1.55 $\pm$ 0.06	1.57 $\pm$ 0.08	1.63 $\pm$ 0.06
Prazosin (0.1 μ M) with AII (0.05 μ M) (<i>n</i> = 6)	1.08 $\pm$ 0.09*	1.32 $\pm$ 0.06	1.67 $\pm$ 0.07

* Represents a significant difference for the log agonist concentration-ratio value for NA calculated at the 25% level of the maximum response in the presence of AII.

any postjunctional α_2 -adrenoceptors by masking the receptor with 1 μ M rauwolscine. This concentration of rauwolscine was considered to be without effect at α_1 -adrenoceptors, since it was without effect on responses to phenylephrine in this preparation.

The inclusion of rauwolscine (1 μ M) prior to, and during, the incubation period with phenoxybenzamine (0.3 μ M), did not result in part of the response to NA being 'spared' (Figure 4).

Figure 4 An examination of contractile responses of the rabbit isolated distal saphenous artery to noradrenaline (NA) following attempted isolation of post-junctional α_2 -adrenoceptors with a combination of (●) 0.3 μ M phenoxybenzamine and 1 μ M rauwolscine, and the effect of 5 nM (■) and 0.05 μ M (□) angiotensin II. Results are expressed as a % of the initial maximum response to (○) noradrenaline. Each point represents the mean of *n* = 6–11; s.e.mean shown by vertical bars. All responses to noradrenaline in the presence of angiotensin II (0.05 μ M) after the protection protocol are significantly different from those after the protection protocol alone, *P* < 0.05, Student's *t* test.

Table 2 List of pD₂ values (with 95% confidence limits) for the α -adrenoceptor agonists, noradrenaline (NA), phenylephrine and UK-14304 under normal experimental conditions (control) and after attempted isolation of postjunctional α_2 -adrenoceptors with the combination of rauwolscine (Rauw, 1 μ M) and phenoxybenzamine (Pbz) (0.3 μ M) in the presence of angiotensin II (AII, 0.05 μ M), in the rabbit isolated distal saphenous artery

	NA	Phenylephrine	UK-14304
Control*	7.25 $\pm$ 0.09 (<i>n</i> = 6)	6.56 $\pm$ 0.08 (<i>n</i> = 5)	6.59 $\pm$ 0.12 (<i>n</i> = 7)
After Rauw/Pbz in the presence of AII (0.05 μ M)	6.66 $\pm$ 0.29 (<i>n</i> = 11)	< 5.15* (<i>n</i> = 6)	8.36 $\pm$ 0.31 (<i>n</i> = 7)

* A precise pD₂ value for phenylephrine has not been calculated since a true maximum response to this agonist was not achieved under these conditions.

* Values from Dunn *et al.* (1989).

Figure 5 Responses to the α -adrenoceptor agonists noradrenaline (NA), (○), phenylephrine (□) and UK-14304 (■) in (a) the absence and (b) the presence of angiotensin II (0.05 μ M) after attempted isolation of postjunctional α_2 -adrenoceptors in the rabbit isolated distal saphenous artery. Results are expressed as a % of the maximum response to NA (○) prior to the protection protocol. Each point represents the mean of *n* = 6–11; vertical bars show s.e.mean.

The maximum response to NA was reduced to a similar degree by phenoxybenzamine alone and by the combination of rauwolscine and phenoxybenzamine (% reduction of NA

Figure 6 The effects of (□) 0.1 μ M prazosin, (■) 1 μ M rauwolscine and a combination of (△) 0.1 μ M prazosin and 1 μ M rauwolscine on responses to noradrenaline (NA), in the presence of 0.05 μ M angiotensin II after attempted isolation of postjunctional α_2 -adrenoceptors (●) in the rabbit isolated distal saphenous artery. Results are expressed as a % of the initial maximum response to NA (○) prior to the protection protocol. Each point represents the mean of *n* = 5–11; vertical bars show s.e.mean.

Figure 7 Contractile responses of the rabbit isolated saphenous artery to the selective α_2 -adrenoceptor agonist UK-14304 in the (○) absence and the (●) presence of (a) the selective α_1 -adrenoceptor agonist phenylephrine (10 nM), (b) 0.03 μ M Bay K 8644, (c) 5-hydroxytryptamine (5-HT, 0.01–0.3 μ M) and (d) KCl (17–20 mM). The 'active tone' induced by phenylephrine, 5-HT and KCl was approximately 15% of the maximum response to noradrenaline. Results are expressed as a % of the initial maximum responses to UK-14304 (○). Each point represents the mean of $n = 5$; vertical bars show s.e.mean.

maximum response: 97.4 ± 1.2 and 95.8 ± 2.3 , respectively). If, however, the preparations were exposed to AII (5 nM–0.05 μ M) after the combination of rauwolscline (1 μ M) and phenoxybenzamine (0.3 μ M), concentration-dependent responses to NA were observed (Figure 4). The maximum response after attempted isolation of postjunctional α_2 -adrenoceptors was increased from 4.2 ± 2.3 to $35.1 \pm 2.9\%$ ($n = 11$) of the initial maximum response to NA in the absence and presence of 0.05 μ M AII, respectively. Similarly, 5 nM AII allowed the expression of responses to NA equivalent to $23.3 \pm 3.35\%$ ($n = 9$) of the initial maximum response before treatment with the combination of rauwolscline and phenoxybenzamine (Figure 4).

AII was also found to be a necessary prerequisite for the expression of responses to the α -adrenoceptor agonists, phenylephrine and UK-14304, after the protection protocol (Figure 5). In the presence of AII all agonists, NA, phenylephrine and UK-14304, produced concentration-dependent contractions (Figure 5b). Under these conditions the relative potency of the agonists was UK-14304 > NA > phenylephrine, in contrast to the potency ratio of NA > phenylephrine = UK-14304 seen under normal experimental conditions (Table 2).

Effects of α -adrenoceptor antagonists

As shown in Figure 6, responses to NA in the presence of AII (0.05 μ M), after attempted isolation of postjunctional α_2 -adrenoceptors, were resistant to prazosin (0.1 μ M) (pD_2 value for NA: 6.66 ± 0.29 and 6.42 ± 0.18 , in the absence and presence of prazosin (0.1 μ M) respectively, $n = 5$), but susceptible to rauwolscline (1 μ M) which produced an approximately 100 fold rightward displacement of the CCRC to NA. Furthermore the combination of prazosin (0.1 μ M) and rauwolscline (1 μ M) was no more effective in antagonizing responses to NA than was rauwolscline (1 μ M) alone (Figure 6).

Effect of other agents on responses to UK-14304

A concentration of phenylephrine selective for postjunctional α_1 -adrenoceptors (10 nM), produced a sustained contraction equivalent to $13.8 \pm 2.0\%$ of the maximum response to UK-14304 in the rabbit isolated distal saphenous artery. Subsequently, exposure of the preparation to UK-14304 resulted in concentration-dependent contractions superimposed upon the induced tone. The sensitivity of the preparation to UK-14304 was markedly increased in a manner analogous to that seen with AII (see: Dunn *et al.*, 1989), with the threshold sensitivity of the preparation to UK-14304 being increased from approximately 0.01 μ M to 0.03 nM in the presence of the α_1 -adrenoceptor agonist-induced tone (Figure 7a).

Bay K 8644 (0.03 μ M) did not alter resting tension in the saphenous artery, but significantly increased responses to UK-14304 in the preparation. This enhancement was associated with an approximately 25% increase in the maximum response and a 2 fold leftward displacement of CCRC to UK-14304 (Figure 7b). However, in spite of the potentiation produced by Bay K 8644, there was no increase in the threshold concentration of UK-14304 for contraction, which was approximately 0.01 μ M in the absence and presence of Bay K 8644. Similar observations were made following induction of 'active-tone' with 5-hydroxytryptamine (5-HT, 0.01–0.3 μ M), yielding a response approximately 15% of the NA maximum (Figure 7c). The presence of comparable 'active-tone' with KCl (17–20 mM) was associated with a significant increase in the sensitivity of the preparation to UK-14304 (Figure 7d). However, this was not as pronounced as that observed with either AII or phenylephrine.

Discussion

Under normal experimental conditions the rabbit isolated distal saphenous artery appears to possess a homogeneous population of postjunctional α_1 -adrenoceptors. Prazosin produced a concentration-dependent rightward displacement of the CCRC to NA, with a pA_2 value of 8.57 and the slope of the Schild plot did not differ significantly from unity, indicating competitive antagonism. This pA_2 value for prazosin is consistent with reported pA_2 values against α_1 -adrenoceptor-mediated responses in other vascular preparations from the rabbit such as the thoracic aorta (8.7)

(Docherty *et al.*, 1981), ear artery (8.6) (Hieble *et al.*, 1982) or pulmonary artery (8.8) (Kaposci *et al.*, 1987). Conversely, rauwolscline ($1\ \mu\text{M}$), at a concentration approximately 100 times greater than that equivalent to its pA_2 value at postjunctional α_2 -adrenoceptors in the rabbit ear vein (Daly *et al.*, 1988b) and its dissociation constant in the lateral saphenous vein after isolation of postjunctional α_2 -adrenoceptors (Daly *et al.*, 1988a), produced only a small rightward displacement of the CCRC to NA in this preparation. The estimated $-\log K_B$ value for rauwolscline (6.32) in the rabbit distal saphenous artery is therefore slightly higher, but not dissimilar to its reported pA_2 value at postjunctional α_1 -adrenoceptors in other rabbit vascular preparations, such as the thoracic aorta (6.1) and ear artery (5.5) (Daly *et al.*, 1988c).

The influence of angiotensin II

In a recent paper we demonstrated that AII uncovered responses to UK-14304 which were mediated via postjunctional α_2 -adrenoceptors in the rabbit isolated distal saphenous artery (Dunn *et al.*, 1989). In the present study, AII did not influence responses to the selective α_1 -adrenoceptor agonist phenylephrine and perhaps surprisingly, was also without effect on responses to the nonselective agonist NA. This peptide did, however, make responses to NA more resistant to the antagonistic action of prazosin, particularly on the lower portion of the CCRC, in effect introducing a prazosin-resistant component of responses to NA. The observations parallel results obtained in the rat tail artery, in which the lower third of the CCRC to NA is slightly 'resistant' to prazosin over a limited prazosin concentration range (Rajanayagam & Medgett, 1987) and in which postjunctional α_2 -adrenoceptor-mediated contractile responses have subsequently been demonstrated (Templeton *et al.*, 1989). Furthermore, AII also reduces the potency of prazosin on responses to both B-HT 920 and NA in the rabbit lateral saphenous vein, another preparation in which postjunctional α_2 -adrenoceptors have been clearly shown (Schümmann & Lues, 1983; Daly *et al.*, 1988a; Dunn *et al.*, 1991). The introduction of a prazosin-resistant component of responses to NA, therefore, strongly indicates the presence of both postjunctional α_1 - and α_2 -adrenoceptors in the distal saphenous artery.

Attempted isolation of postjunctional α_2 -adrenoceptors in rabbit isolated distal saphenous artery

Phenoxybenzamine ($0.3\ \mu\text{M}$), an irreversible α -adrenoceptor antagonist (Furchgott, 1972), virtually abolished responses to NA in the rabbit isolated distal saphenous artery. The basis for receptor isolation, therefore, was to provide conditions whereby postjunctional α_2 -adrenoceptors could be selectively protected by the inclusion of $1\ \mu\text{M}$ rauwolscline in a manner analogous to that used in the rabbit isolated lateral saphenous vein (Daly *et al.*, 1988a). This concentration of α_2 -adrenoceptor antagonist was considered to be selective since it was without effect on responses to phenylephrine in the distal saphenous artery.

In contrast to the rabbit lateral saphenous vein (see Daly *et al.*, 1988a), the inclusion of rauwolscline ($1\ \mu\text{M}$) prior to and during exposure to phenoxybenzamine, did not result in part of the response to NA being spared from the action of phenoxybenzamine. In the presence of AII, however, concentration-dependent responses were observed to the α -adrenoceptor agonists UK-14304, NA and phenylephrine. The rank order of potencies for these agonists, UK-14304 > NA > phenylephrine differs from the potency order seen under normal circumstances, NA > phenylephrine > UK-14304 (Dunn *et al.*, 1989) and is similar to the potency order for agonists found in the rabbit ear vein, which contains predominantly postjunctional α_2 -adrenoceptors (Daly *et al.*, 1988b).

Additionally, responses to NA after the receptor protection protocol in the presence of AII were resistant to $0.1\ \mu\text{M}$ prazosin, a concentration approximately 30 times greater than for its pA_2 value on postjunctional α_1 -adrenoceptors in this preparation. Furthermore, rauwolscline ($1\ \mu\text{M}$) produced an approximately 100 fold rightward displacement of the CCRC to NA, yielding an estimated $-\log K_B$ value of 8.01. This value corresponds well with that obtained against responses to NA in the lateral saphenous vein after isolation of postjunctional α_2 -adrenoceptors (Daly *et al.*, 1988a) and against responses to NA in the rabbit ear vein (Daly *et al.*, 1988b). Further evidence favouring the existence of a homogeneous population of postjunctional α_2 -adrenoceptors, under these conditions, is provided from the observation that the combination of prazosin ($0.1\ \mu\text{M}$) and rauwolscline ($1\ \mu\text{M}$) was no more effective in inhibiting responses to NA than was rauwolscline ($1\ \mu\text{M}$) alone. Essentially these results are similar to those obtained under normal experimental conditions, where the presence of AII was a prerequisite for the demonstration of prazosin-resistant responses to both UK-14304 (see: Dunn *et al.*, 1989) and NA (present study).

Interaction between postjunctional α_1 - and α_2 -adrenoceptors

A number of observations in the rabbit isolated distal saphenous artery suggest that postjunctional α_1 - and α_2 -adrenoceptors do not co-exist in a simple manner. For example, despite the fact that functional postjunctional α_2 -adrenoceptors can be clearly demonstrated in this preparation, under appropriate conditions, no component of the response to NA is resistant to prazosin when both α -adrenoceptors are present. In addition, having clearly demonstrated a facilitatory role for AII on responses mediated via postjunctional α_2 -adrenoceptors, we may then have expected this agent to enhance responses to NA, a non-selective agonist, when the full receptor complement was present. Such an action was not observed.

These apparently paradoxical observations could be reconciled if the two postjunctional α -adrenoceptors 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 in the distal saphenous artery may be dependent upon simultaneous stimulation of the α_1 -adrenoceptor population. With the latter predominating, this would explain the relatively high potency of prazosin, with no component of responses to NA (or other agonists) exhibiting resistance to the α_1 -adrenoceptor antagonist, and the relatively low potency of rauwolscline. Since both α -adrenoceptors are present under normal experimental conditions, the positive influence required for the expression of α_2 -adrenoceptor-mediated responses would be provided by stimulation of postjunctional α_1 -adrenoceptors. Assuming a similar mechanism to be involved, the requirement for AII would therefore be superfluous, explaining the lack of a facilitatory action for this agent against responses to NA when the full α -adrenoceptor complement was active.

If, however, α_1 -adrenoceptors are inhibited, by either prazosin or phenoxybenzamine, a positive influence for AII would be a prerequisite for the expression of α_2 -adrenoceptor-mediated responses. This would explain the induction of prazosin-resistance produced by AII, and the requirement for the presence of this agent to produce responses to NA mediated via postjunctional α_2 -adrenoceptors, after irreversible inactivation of postjunctional α_1 -adrenoceptors with phenoxybenzamine. An interaction between the α -adrenoceptors in this preparation was directly demonstrated by inducing a small degree of tone in the distal saphenous artery with a selective concentration of phenylephrine ($0.01\ \mu\text{M}$). Stimulation of α_1 -adrenoceptors resulted in the introduction of a previously 'unseen' component of responses to UK-14304, at very low concentrations,

analogous to that introduced by AII (see Dunn *et al.*, 1989). Attempting to examine the effects of α -adrenoceptor antagonists on these responses would have negated the experiment. However, it is reasonable to assume that the uncovered responses to UK-14304 were mediated via postjunctional α_2 -adrenoceptors. Similarly, phenylephrine-induced tone has been shown to uncover responses to B-HT 920 in the canine portal vein (Furuta, 1988). The concept of interacting postjunctional α -adrenoceptors in the distal saphenous artery is represented schematically in Figure 8.

A similar interaction between postjunctional α -adrenoceptors has previously been implicated in the rabbit lateral saphenous vein (Daly *et al.*, 1988d) although the dependency of α_2 -adrenoceptor-mediated responses on α_1 -adrenoceptor stimulation is somewhat less. This is apparent since NA can clearly sustain a response alone after isolation of postjunctional α_2 -adrenoceptors (Daly *et al.*, 1988a), contrasting with the distal saphenous artery, where responses are wholly dependent on the presence of a stimulatory agent. Again in this preparation, the facilitatory action of AII on postjunctional α_2 -adrenoceptor-mediated responses is apparent only if α_1 -adrenoceptors are blocked by prazosin or phenoxybenzamine (Dunn *et al.*, 1991). An even more subtle interaction between α_1 - and α_2 -adrenoceptors has been demonstrated in the cat nictitating membrane (Shepperson, 1984) and rat isolated tail artery (Xiao & Rand, 1989). In these preparations, 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 produce a functional response. This effect could be blocked by selective α_2 -adrenoceptor antagonists.

Figure 8 Diagrammatic representation of the proposed interaction between postjunctional α -adrenoceptor subtypes in the rabbit isolated distal saphenous artery. Note that under normal circumstances (a), stimulation of postjunctional α_1 -adrenoceptors provides the positive input required for the expression of postjunctional α_2 -adrenoceptors thereby rendering all responses sensitive to prazosin. Such a positive influence for angiotensin II (AII) is only necessary if α_1 -adrenoceptors are blocked with either prazosin (Praz) or phenoxybenzamine (Pbz) (b).

Since the original demonstration of postjunctional α_2 -adrenoceptors in the pithed rat (Drew & Whiting, 1979; Docherty *et al.*, 1979), identification of this subtype in isolated vascular preparations, both responding to NA and resistant to prazosin or a suitable α_1 -adrenoceptor antagonist, has proved very difficult. While a number of explanations have been put forward to explain the elusive nature of this receptor subtype (see McGrath *et al.*, 1989), it is clear from the present study that one such factor that may conspire to render contractions mediated by postjunctional α_2 -adrenoceptors 'prazosin-sensitive', is the dependency of α_2 -adrenoceptor-mediated contractions on stimulation of α_1 -adrenoceptors. Since other contractile agents can substitute for the positive influence of α_1 -adrenoceptors, prazosin-resistant responses could be easily demonstrated in whole animals due to the presence of circulating vasoactive substances. In this respect, AII would assume great importance, particularly in models such as the pithed rat where the renin-angiotensin system is highly activated (Grant & McGrath, 1988).

Such an interaction between postjunctional α -adrenoceptors has widespread implications for the characterization of the α -adrenoceptor populations in blood vessels currently available for study *in vitro*. This is perhaps best illustrated by two further examples, which suggest that the clear demonstration of α_2 -adrenoceptor-mediated responses has been obscured by their indirect sensitivity to prazosin.

Firstly, responses to NA in the isolated vascular bed of the rat tail are prazosin-sensitive, rauwolscine-resistant (α_1 -mediated) under normal experimental conditions, but in contrast, a sizeable component of the response to NA becomes prazosin-resistant, rauwolscine-sensitive (α_2 -mediated) in the presence of vasopressin-induced tone (Templeton *et al.*, 1989). Similarly, inducing tone with phenylephrine or endothelin also introduced rauwolscine-sensitive responses to UK-14304 in this preparation (Templeton, 1989; MacLean & McGrath, 1990). Secondly, responses to NA in the rabbit femoral artery were shown to be prazosin-sensitive, rauwolscine-resistant, and therefore by definition α_1 -adrenoceptor-mediated (Purdy & Weber, 1988). AII also introduces a component of responses to UK-14304 in the femoral artery in an analogous manner to that seen in the distal saphenous artery (authors unpublished observations). It would seem reasonable, therefore, to predict that further analysis would demonstrate unequivocally the presence of postjunctional α_2 -adrenoceptors in the rabbit femoral artery.

Thus, the widespread use of a single concentration of an α_1 -adrenoceptor antagonist (usually prazosin), as a basis for determining the characteristics of a population of α -adrenoceptors is no longer acceptable. Furthermore, any interaction between α_1 - and 'silent' α_2 -adrenoceptors has major implications for the use of phenoxybenzamine for inferential determination of agonist affinity (Furchgott, 1972). This irreversible antagonist possesses selectivity for vascular α_1 -adrenoceptors (Constantine *et al.*, 1982; Dunn *et al.*, 1991), and a concentration that eliminates any 'receptor-reserve' (viewed as a reduction in the maximum response) will inevitably alter the relative contribution of α_1 - and α_2 -adrenoceptors to the observed response before and after inactivation. Hence, one of the underlying assumptions for the use of phenoxybenzamine in the quantitative analysis of the interaction between agonist and α -adrenoceptor (see: Besse & Furchgott, 1976) cannot be guaranteed in vascular smooth muscle.

Such an interaction between postjunctional α_1 - and α_2 -adrenoceptors may also partially explain the wide disparity in the relative potencies of α_1 - and α_2 -adrenoceptor antagonists found between vascular preparations (see Drew, 1985; McGrath *et al.*, 1989). If, for example, the expression of postjunctional α_2 -adrenoceptors is completely dependent upon α_1 -adrenoceptor stimulation, then we may expect prazosin potentially to antagonize responses (as is the case in the distal saphenous artery). The potency of α_2 -adrenoceptor antagonists will, however, depend upon the relative predominance of this subtype. Therefore, with an increasing contribution of

postjunctional α_2 -adrenoceptors to the total response, the pA_2 values for α_2 -adrenoceptor antagonists will increase accordingly. It is interesting to note that in the human femoral artery and canine portal vein, in both of which there is some evidence suggesting the presence of postjunctional α_2 -adrenoceptors (Glusa & Markwardt, 1983; Furuta, 1988), prazosin is only 11–35 fold more potent than yohimbine (De Mey & Vanhoutte, 1981; Shoji *et al.*, 1983). Similarly in the canine isolated splenic artery and vein the rauwolscine and prazosin potency ratio against noradrenaline (both agents produced competitive antagonism) was 12 and 3, respectively (Hieble & Woodward, 1984). This stands in contrast to the rabbit pulmonary artery (Starke, 1981), rat aorta or portal vein (Digges & Summers, 1983) where the differential is greater than 200 fold.

In summary, despite the fact that prazosin competitively antagonizes responses in the rabbit isolated distal saphenous artery and that no component of the CCRC to NA is resistant

to the α_1 -adrenoceptor antagonist, responses to NA mediated via postjunctional α_2 -adrenoceptors can be demonstrated in this preparation under the appropriate experimental conditions. The susceptibility to prazosin of responses to NA can be explained by a functional interaction between the α -adrenoceptor subtypes, such that responses mediated via postjunctional α_2 -adrenoceptors are dependent upon a degree of stimulation of α_1 -adrenoceptors. In the presence of α_1 -adrenoceptor blockade the necessary positive influence for α_2 -adrenoceptor expression can be provided by stimulation of AII receptors.

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