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An examination of the postjunctional α-adrenoceptor subtypes for (−)-noradrenaline in several isolated blood vessels from the rabbit

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1 Postjunctional α-adrenoceptors in several isolated blood vessels from the rabbit have been characterized on the basis of the relative potency of the agonists noradrenaline (NA, non-selective), phenylephrine (α_1-selective) and UK-14304 (α_2-selective), and the potency of antagonists rauwolscine (α_2-selective) and corynanthine (α_1-selective) against contractions elicited by NA. In addition, the potency of prazosin against NA was also assessed in the venous preparations.

2 The thoracic aorta, ear artery and left renal vein appear to possess α_1-adrenoceptors since the agonist potency order was NA > phenylephrine > UK-14304, while corynanthine was 3–10 fold more potent than rauwolscine.

3 The ear vein appears to possess α_2-adrenoceptors. The rank order of agonist potency was UK-14304 > NA ≫ phenylephrine and all three agonists elicited responses of similar magnitude. Furthermore, rauwolscine was 30 fold more potent than corynanthine while prazosin failed to produce a concentration-dependent inhibition.

4 The saphenous vein and the plantaris vein appear to possess a mixture of both subtypes since the rank order of agonist potency was UK-14304 > NA ≫ phenylephrine, while responses elicited by UK-14304 were smaller than those to the other agonists. However, although rauwolscine was 20 to 100 fold more potent than corynanthine in both preparations, suggestive of predominantly α_2-adrenoceptors, prazosin was either potent (saphenous vein) or relatively inactive (plantaris vein).

5 The characteristics of postjunctional α_1- and α_2-adrenoceptors on isolated blood vessels from the rabbit are discussed in relation to the value of both the agonists, particularly NA, and the antagonists used in this study.

Introduction

Furchgott (1972) defined an adrenoceptor in the following terms 'a wide variety of tissues undergo a change of functional state on exposure to noradrenaline or adrenaline. Those molecular constituents of the effector cells of a tissue with which these molecules must first interact in order to produce a change of state – or response – of the tissue, are the so-called adrenoceptors'. Central to this definition of what constitutes an adrenoceptor are the catecholamines.

The first evidence that suggested that more than one population of postjunctional α-adrenoceptors may exist on arterial smooth muscle was based upon the observation that pressor responses to (−)-noradrenaline (NA) in pithed rats and cats were resistant to the α-adrenoceptor antagonist prazosin but sensitive to phentolamine (Bentley *et al.*, 1977; Drew & Whiting, 1979). With a few notable exceptions (e.g. Flavahan & McGrath, 1980; Wilffert *et al.*, 1982), the majority of the subsequent *in vivo* studies that provided much of the evidence for the presence of postjunctional α_1- and α_2-adrenoceptors on vascular smooth muscle (McGrath, 1982), relied upon the use of putative 'selective' agonists that bear no structural similarity to the catecholamines, NA and (−)-adrenaline.

In contrast to the relative ease of demonstrating postjunctional α_2-adrenoceptors *in vivo*, identification of this subtype with 'selective agonists' *in vitro* has been extremely difficult (McGrath, 1982; Shoji *et al.*, 1983; Flavahan *et al.*, 1984). Part of the problem clearly lies with the 100 fold potency range found in the literature for the standard selective α_1-adrenoceptor antagonist prazosin (Drew, 1985), resistance to which is an indicator of the presence of α_2-adrenoceptors (McGrath, 1982). This factor is further compounded by a less pronounced variation in the potency of the α_2-adrenoceptor antagonist yohimbine at postjunctional α_1-adrenoceptors (Agrawal *et al.*, 1985).

With these particular problems in mind, we have attempted to examine the postjunctional α-adrenoceptors in several isolated blood vessels from the rabbit with the aim of providing simple qualitative 'rules of thumb' for the identification of the α_2-subtype. This was undertaken in two ways. First, by comparing the relative potency and intrinsic activity of three agonists: NA, the selective α_1-adrenoceptor agonist, phenylephrine (McGrath, 1982) and the selective α_2-adrenoceptor agonist UK-14304 (Cambridge, 1981). Secondly, since stereoisomers of antagonists have previously been used as powerful tools in the study of adrenoceptors (see: Fuder *et al.*, 1981), we also compared the relative antagonist potency of the yohimbine diasteroisomers corynanthine (selective for α_1-adrenoceptors) and rauwolscine (selective for α_2-adrenoceptors) against NA.

Methods

White albino New Zealand rabbits of either sex weighing 2.3–3.3 kg were killed by stunning followed by exsanguination. Segments of the thoracic aorta, the ear artery, the left renal vein, the ear vein, the lateral saphenous vein and the lateral planataris vein were cleaned of fat and connective tissue *in situ* and then placed in ice-cold physiological salt solution (PSS). The ear vein was taken as the 10 mm region either side of the first bifurcation of the vein running parallel to the ear artery, while the plantaris vein was taken as the 10 mm distal segment of the continuation of the lateral saphenous vein measured from the ankle. When necessary, preparations were cleaned further with the aid of a dissecting microscope. The lateral plantaris vein: 3 mm length segments were taken from each vein (5 mm for the ear vein) or artery 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. The preparations were then mounted in 30 ml isolated organ baths under an initial resting tension of 4 g (aorta), 2.5 g (ear artery), 0.5 g (renal vein), 0.3 g (ear vein), 1.5 g (lateral plantaris vein), 2 g (lateral saphenous vein) and allowed to relax. The endothelium of both the aorta and the ear artery were removed by gentle rubbing of the intimal surface of the vessels with the edge of a pair of forceps. Venous preparations were not rubbed. 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 was achieved, each preparation was exposed to 3 μM NA and allowed to contract for 10 min. Complete removal of the endothelium in arterial preparations was confirmed by the inability of 1 μM acetylcholine to effect a relaxation of responses to 3 μM NA in rubbed preparations. 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 remained stable for the rest of the experiment; 1.5–2.0 g (aorta); 1.0–1.5 g (ear artery); 0.15–0.2 g (renal vein); 0.075–0.1 g (ear vein); 0.3–0.4 g (lateral plantaris vein, lateral saphenous vein). 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 to the previous concentration. In the majority of venous 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 two experimental protocols adopted.

(1) Cumulative concentration-response curves to phenylephrine and UK-14304 were constructed (the order was alternated in individual experiments) and responses expressed as a percentage of both the maximum response to NA and of its 'own' maximum. pD_2 values (the $-\log$ of the concentration required to produce 50% of its own maximum) were calculated for each agonist (van Rossum, 1963).

(2) Preparations were exposed to various concentrations of an antagonist for a minimum of 45 min and the agonist CRC repeated. A maximum of three curves was generated in each preparation (two in the presence of an antagonist). Preliminary experiments

with preparations not exposed to antagonists established that for the aorta, ear artery, ear vein and renal vein, time-dependent changes in the sensitivity of the preparations were small (<0.15 log concentration unit) and, because of the comparative nature of the study with the antagonists rauwolscine and corynanthine, time-controls were not routinely employed for these preparations. However, preparations from the lateral saphenous vein and the lateral plantaris vein were subject to variable changes in agonist sensitivity (<0.23 log concentration unit), thus necessitating time-control preparations in each experiment.

According to Arunlakshana & Schild (1959) if antagonism is competitive, a plot of the logarithm of the (agonist concentration-ratio −1) against the 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. pA_2 values and the slope of the Schild plot were determined by linear regression and are expressed with the 95% confidence limits. With the exception of the effect of rauwolscine on NA-induced contractions in the lateral saphenous vein, which was determined at the 75% level of the maximum response, agonist concentration-ratios in all preparations were determined at the level of 50% of the maximum response.

All other responses are expressed as a percentage (mean ± s.e.mean) of the maximum response of the first concentration-response curve. Differences between means were considered statistically different if $P < 0.05$ for either paired or unpaired observations (Student's t test).

The composition of the PSS was (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4 . 7H_2O$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2 and glucose 11.1. Na_2 EDTA 23 μM 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. For the two arterial preparations, 30 μM corticosterone was included to inhibit extraneuronal uptake as this route of elimination has important consequences for α-adrenoceptor function (Henseling, 1983; Johnson & De La Lande, 1978). However, a detailed examination of the role of extraneuronal uptake on α-adrenoceptor function in the isolated lateral saphenous vein failed to reveal a significant role (Daly *et al.*, 1988b), and thus, an uptake$_2$ inhibitor was not included. Furthermore, Guimaraes & Paiva (1981) have shown that, for the dog isolated saphenous vein, extraneuronal uptake exerts a greater influence on β-adrenoceptor function than on α-adrenoceptor function and thus, from a theoretical point of view, inhibition of uptake$_2$ could compromise the conditions required for the examination of the characteristics of α-adrenoceptors with NA.

The following drugs were used: (−)-noradrenaline bitartrate (Sigma), phenylephrine HCl (Sigma), prazosin HCl (Pfizer), corynanthine (HCl (Roth), rauwolscine HCl (Roth), (±)-propranolol HCl (Sigma) and cocaine HCl (Macarthys), UK-14304 bitartrate (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline; Pfizer), corticosterone (Sigma). With the exception of corticosterone, which was dissolved in propylene glycol, all drugs were dissolved in distilled water and added to the organ baths in a volume of 0.15 ml or less.

Results

Thoracic aorta, ear artery and left renal vein

Figure 1 shows the effects of NA, phenylephrine and UK-14304 in these three preparations, relative to the maximum response elicited by NA. Phenylephrine elicited maximum contractions comparable to NA in the thoracic aorta and ear artery and slightly smaller responses than NA in the left renal vein. In marked contrast, maximum contractions to UK-14304 were smaller than those to either NA or phenylephrine in the ear artery or thoracic aorta and failed to elicit any response in 4 out of 5 preparations of the left renal vein. NA was approximately 5 fold more potent than phenylephrine in all three preparations and between 50 and 100 fold more potent than UK-14304 in the ear artery and thoracic aorta (Table 1).

Corynanthine (1 μM–50 μM) produced a concentration-dependent rightward displacement of the NA concentration-response curve (CRC) in all three preparations without a change in the slope of the CRC. Since the confidence limits for the slope of the Schild plot overlapped unity this inhibition was competitive (Table 2). pA_2 values in the three preparations varied over a 10 fold range, though this may be a reflection of the differences in the slope of the Schild plots (cf: ear artery and left renal vein). Rauwolscine (2.5 μM–50 μM) also produced a concentration-dependent rightward shift of the NA CRC in all three preparations without changing either the slope or the maximum response. However, based upon the slope of the Schild plot, this was non-competitive in the ear artery (Table 2). In each preparation, corynanthine was 8–30 fold more potent than rauwolscine against NA-induced contractions.

Ear vein

In the isolated ear vein, NA, phenylephrine and UK-14304 acted as full agonists (Figure 2a) with UK-14304 approximately 4 fold more potent than NA

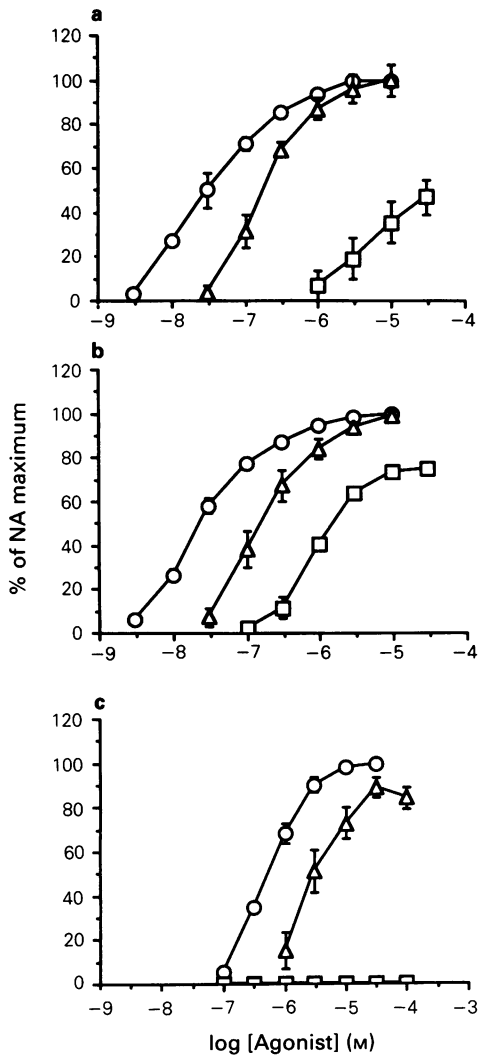

Figure 1 The effects of (−)-noradrenaline (NA) (○), phenylephrine (△) and UK-14304 (□) on the rabbit isolated aorta (a), ear artery (b) and left renal vein (c). All responses are expressed as a percentage of the maximum response to (−)-NA in each preparation and the points shown represent the mean of observations from 4–5 rabbits. The vertical lines indicate the s.e.mean.

and 400 fold more potent than phenylephrine (Table 1). Both corynanthine (0.5 μM–50 μM) and rauwolscine (0.05 μM–10 μM) produced a concentration-dependent parallel rightward displacement of the NA CRC and, with the exception of 10 μM rauwolscine, this was not associated with a reduction in the maximum response (Figure 2c,d). The slope of the Schild plot for both antagonists (for this analysis rauwolscine was examined over only a 50 fold concentration range, 0.05 μM–2.5 μM) was not significantly different from unity and, therefore, the interaction was competitive. As judged by a comparison of pA_2 values, rauwolscine was approximately 30 fold more potent than corynanthine.

In marked contrast to both corynanthine and rauwolscine, prazosin (0.1 μM and 1 μM) did not effect a concentration-dependent inhibition of responses to NA (Figure 2b).

Lateral plantaris vein

In the isolated lateral plantaris vein, UK-14304 produced approximately 60% of the maximum contraction effected by both NA and phenylephrine, but was 3 fold more potent than NA and 70 fold more potent than phenylephrine (Figure 3a; Table 1). Both corynanthine (0.5 μM–50 μM) and rauwolscine (0.5 μM–50 μM) effected a concentration-dependent rightward parallel displacement of the NA CRC without markedly reducing the maximum response (Figure 3b,d). The 95% confidence limits for the slope of the Schild plots were not significantly different from unity and, based upon the pA_2 values, rauwolscine was approximately 20 fold more potent than corynanthine (Table 2).

Figure 3c shows the effect of prazosin on contractions elicited by NA in the lateral plantaris vein; 0.01 μM (not shown), 0.1 μM and 1 μM prazosin produced a concentration-dependent shift of the upper third of the NA CRC without changing the maximum response, while the lower part of the CRC was much less affected. Thus, prazosin caused a marked change in the slope of the NA CRC. The combination of 2.5 μM rauwolscine and 1 μM prazosin resulted in a CRC to NA which was parallel to the original control CRC, i.e.: a marked rightward displacement of the lower portion of the CRC without a significant change in the upper third compared to that in the presence of 1 μM prazosin alone.

Lateral saphenous vein

In the isolated lateral saphenous vein, UK-14304 produced a maximum response that amounted to approximately 80% of that produced by either NA or phenylephrine, but was 2 fold more potent than NA and 100 fold more potent than phenylephrine (Figure 4a; Table 1). Corynanthine (0.5 μM–12.5 μM) produced a concentration-dependent rightward parallel displacement of the NA CRC without reducing the maximum responses (Figure 4b). The slope of the Schild plot was not significantly different from unity (Table 2). In marked contrast, rauwolscine (0.05 μM–2.5 μM) produced a non-parallel

Table 1 A comparison of the effect of (−)-noradrenaline (NA), phenylephrine and UK-14304 at α-adrenoceptors in several isolated blood vessels from the rabbit

		Max response to (−)-NA (g)	*Intrinsic activity*	*pD_2*	*Potency relative to (−)-NA*
Thoracic aorta	(−)-NA	5.6 ± 0.26	1	7.47 ± 0.07	
(*n* = 6)	Phenylephrine		1	6.80 ± 0.06	0.21
	UK-14304		0.48	5.33 ± 0.18	0.007
Ear artery	(−)-NA	6.1 ± 0.59	1	7.67 ± 0.07	
(*n* = 4)	Phenylephrine		1	6.80 ± 0.12	0.13
	UK-14304		0.75	6.04 ± 0.06	0.02
Renal vein	(−)-NA	1.20 ± 0.2	1	6.30 ± 0.06	
(*n* = 4)	Phenylephrine		0.9	5.45 ± 0.18	0.14
	UK-14304		0		
Ear vein	(−)-NA	0.40 ± 0.07	1	7.53 ± 0.08	
(*n* = 4)	Phenylephrine		1.1	5.54 ± 0.06	0.01
	UK-14304		1	8.11 ± 0.16	3.8
Lateral saphenous vein	(−)-NA	4.46 ± 0.35	1	7.53 ± 0.10	
(*n* = 7)	Phenylephrine		1	5.83 ± 0.06	0.02
	UK-14304		0.86	7.85 ± 0.10	2
Lateral plantaris vein	(−)-NA	3.81 ± 0.44	1	6.98 ± 0.06	
(*n* = 5)	Phenylephrine		0.95	5.81 ± 0.06	0.07
	UK14304		0.63	7.58 ± 0.09	3.9

Table 2 pA_2 values and slope of the Schild plots (with 95% confidence limits – except those values taken from other studies) for corynanthine, rauwolscine and prazosin against contractions elicited by (−)-noradrenaline (NA) in several isolated blood vessels from the rabbit

	Corynanthine	*Rauwolscine*	*Prazosin*	*Rauwolscine/coryanthine potency ratio*
Thoracic aorta	7.06 (7.40–6.72)	6.09 (6.37–5.80)	8.7 (a)	9.33
	0.93 (1.07–0.79)	1.02 (1.23–0.8)		
	n = 12	*n* = 12		
Ear artery	7.23 (7.85–6.59)	5.54 (5.65–5.41)	8.6 (b)	48.9
	0.81 (1.01–0.64)	1.34 (1.52–1.14)*		
	n = 12	*n* = 12		
Left renal vein	6.41 (6.73–6.09)	5.68 (5.94–5.45)	8.28 (c)	5.4
	1.03 (1.24–0.81)	0.99 (1.25–0.75)		
	n = 12	*n* = 12		
Ear vein	6.22 (6.45–5.99)	7.70 (7.94–7.45)	NP	0.033
	0.92)1.07–0.76)	1.08 (0.91–1.25)		
	n = 12	*n* = 16		
Lateral saphenous vein	6.32 (6.54–6.16)	8.46 (8.89–8.24)	8.44 (8.72–8.18)	0.008
	0.89 (1.06–0.71)	0.85 (0.96–0.74)*	0.58 (0.68–0.51)*	
	n = 22	*n* = 20	*n* = 56	
Lateral plantaris vein	6.32 (6.66–5.97)	7.56 (8.31–6.8)	NP	0.057
	0.94 (1.18–0.71)	0.80 (1.03–0.56)		
	n = 12	*n* = 18		

Each value is derived from 12–56 individual determinations at a minimum of three concentrations.
* Slope of Schild plot significantly different from unity.
NP Schild analysis not possible due to lack of effect.
(a) Value taken from Docherty *et al.*, (1981).
(b) Value taken from Hieble *et al.*, (1982).
(c) Value taken from Schultz & Westfall (1982).

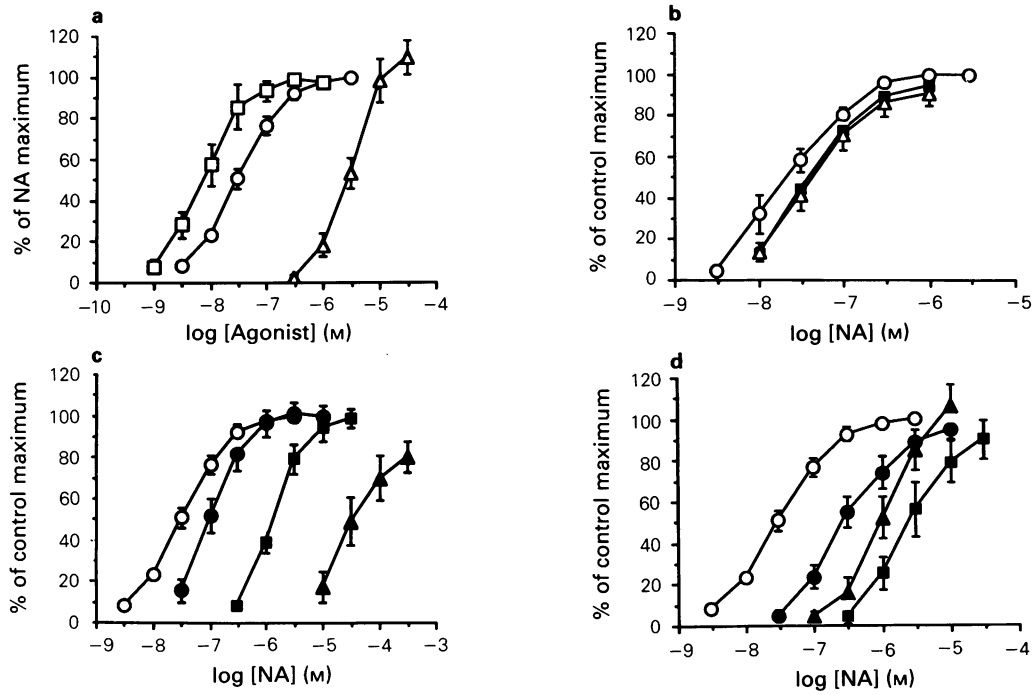

Figure 2 A comparison of the effects of various agonists and antagonists at α-adrenoceptors in the rabbit isolated ear vein. (a) The effect of the agonists (−)-noradrenaline (NA) (○), phenylephrine (△) and UK-14303 (□). (b) The effect of 0.1 μM (△) and 1 μM (■) prazosin on contractions elicited by (−)-NA (○). (c) The effect of 0.05 μM (●), 0.5 μM (■) and 10 μM (▲) rauwolscine on contractions elicited by (−)-NA (○). (d) The effect of 2.5 μM (●), 10 μM (▲) and 50 μM (■) corynanthine on contractions elicited by (−)-NA (○). All responses are expressed as a percentage of either the maximum response to (−)-NA (a) or the maximum response in the control concentration-response curve (b,c,d) and are the mean of a 4–9 observations in different animals. The vertical lines indicate the s.e.mean.

concentration-dependent rightward displacement of the NA CRC—approximately 30% of the lower part of the concentration-response curve was unaffected by these concentrations of rauwolscine (Figure 4d). Based upon the agonist concentration-ratio determined at the 75% level of the maximum response, the antagonism was non-competitive but rauwolscine was approximately 100 fold more potent than corynanthine (Table 2).

Prazosin (0.03 μM–3 μM) produced a concentration-dependent rightward displacement of the NA CRC with no evidence for a reduction in the maximum response except perhaps at 3 μM prazosin (Figure 4c). The 95% confidence limits for the slope of the Schild plot did not overlap unity and thus, the inhibition was non-competitive (Table 2).

Discussion

As indicated in the Introduction, the purpose of the present study was to provide a qualitative pharmacological guide for the identification of the major postjunctional α-adrenoceptor subtype in an isolated blood vessel. To date, this has been largely attempted through the use of the selective α_1-adrenoceptor antagonist prazosin against agonists with reported selectivity for each subtype and, on the whole, this has not been particularly successful in the identification of functional examples of postjunctional α_2-adrenoceptors. In the present study, the agonists and the antagonists employed have provided evidence for three distinct profiles for the α-adrenoceptor population in the isolated blood vessels from the rabbit. This can be best summarized as: (1) Thoracic aorta, ear artery and left renal vein: agonist potency, NA > phenylephrine > UK-14304; intrinsic activity, NA = phenylephrine > UK-14304; antagonists, corynanthine > rauwolscine. (2) Ear vein: agonist potency, UK-14304 > NA ≫ phenylephrine; intrinsic activity, NA = phenylephrine = UK-14304; antagonists, rauwolscine > corynanthine. (3) Lateral saphenous vein and lateral plantaris vein: agonist potency, UK-14304 > NA ≫ phenylephrine;

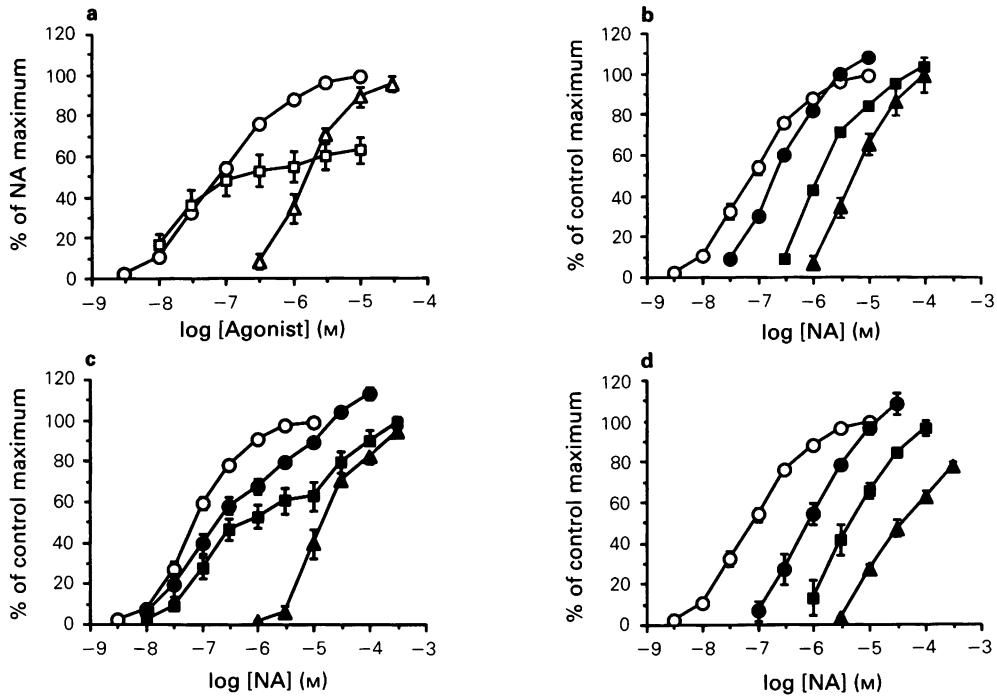

Figure 3 A comparison of the effect of various agonists and antagonists at α-adrenoceptors in the rabbit isolated lateral plantaris vein. (a) The effect of the agonists (−)-noradrenaline (NA) (○), phenylephrine (△) and UK-14304 (□). (b) The effect of 0.5 μM (●), 10 μM (■) and 50 μM (▲ corynanthine on contractions elicited by (−)-NA (○). (c) The effect of 0.1 μM (●) and 1 μM (■) prazosin and a combination of 0.1 μM prazosin and 2.5 μM rauwolscine (▲) on contractions elicited by (−)-NA (○). (d) The effect of 0.5 μM (●), 10 μM (■) and 50 μM (▲) rauwolscine on contractions elicited by (−)-NA (○). All responses are expressed as a percentage of either the maximum response to (−)-NA (a) or the maximum response in the control concentration-response curve (b,c,d) and are the mean of a 4–9 observations in different animals. The vertical lines indicate the s.e.mean.

intrinsic activity, NA = phenylephrine > UK-14304; antagonists, rauwolscine > corynanthine.

In view of the known selectivity of these compounds for α_1-adrenoceptors (phenylephrine and corynanthine) and α_2-adrenoceptors (UK-14304 and rauwolscine) at pre- and post-junctional sites in the rabbit and other species (Weitzell *et al.*, 1979; Cambridge, 1981; McGrath, 1982), the postjunctional α-adrenoceptors in each blood vessel can be identified as follows; the thoracic aorta, ear artery and left renal vein (Group 1) – an α_1 subtype; the ear vein (Group 2) – an α_2-subtype; the lateral saphenous vein and lateral plantaris vein (Group 3) – a mixture of the α_1 and α_2-subtypes, with the latter predominating.

The value of corynanthine and rauwolscine

Central to this study has been the demonstration that the relative potencies of the yohimbine diastereoisomers, corynanthine and rauwolscine, can be used to describe the pharmacological characteristics of the major postjunctional α-adrenoceptor subtype stimulated by NA in each isolated blood vessel. As a general 'rule of thumb', α_1-adrenoceptors (as described by the relative potency of the agonists) are characterized by a 10 fold greater potency of corynanthine, while rauwolscine exerts a 20–100 fold greater antagonism than corynanthine at α_2-adrenoceptors. Clearly, the resolving power of the two diastereoisomers resides in the selectivity of rauwolscine for the α_2-subtype, since in absolute terms (pA_2) corynanthine is as potent at α_1-adrenoceptors in the renal vein as it is at α_2-adrenoceptors in the ear vein (Table 2; Figure 5). However, the low potency of corynanthine at α_1-adrenoceptors in the renal vein, relative to that observed at α_1-adrenoceptors in the aorta and ear artery (Table 2; Figure 5) requires further study. It may be a consequence of the presence of the endothelium and an intact extraneuronal metabolizing system in the

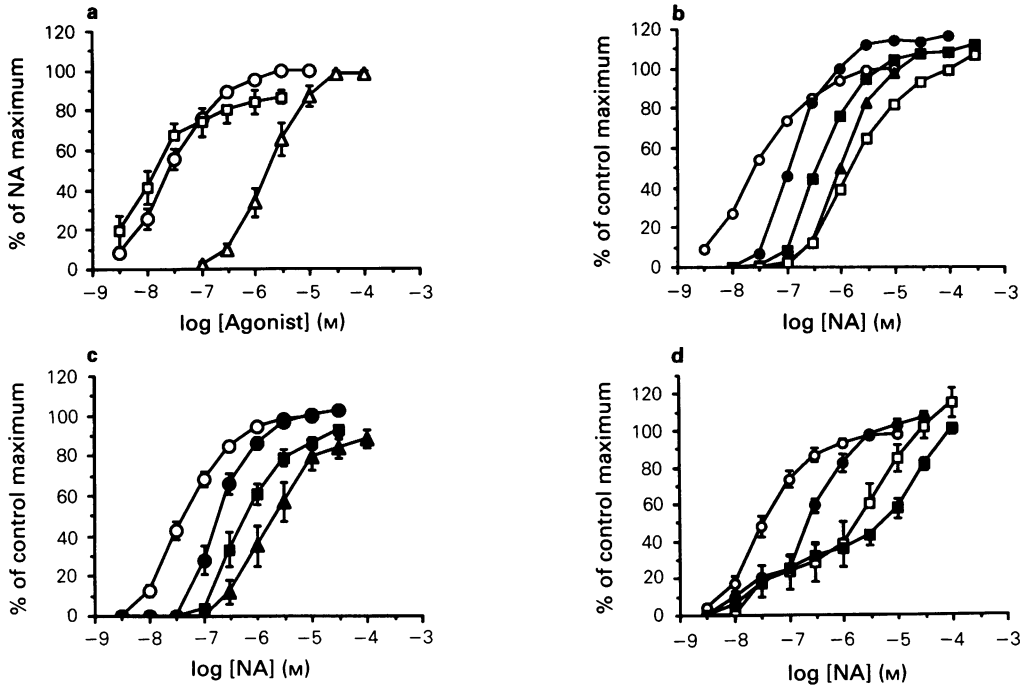

Figure 4 A comparison of the effect of various agonists and antagonists at α-adrenoceptors in the rabbit isolated lateral saphenous vein. (a) The effect of the agonists (−)-noradrenaline (NA) (○), phenylephrine (△) and UK-14304 (□). (b) The effect of 0.5 μM (●), 2.5 μM (■) 10 μM (▲) and 50 μM (□) corynanthine on contractions elicited by (−)-NA (○). (c) The effect of 0.01 μM (●), 0.1 μM (□) and 1 μM (▲) prazosin on contractions elicited by (−)-NA (○). (d) The effect of 0.05 μM (●), 0.5 μM (□) and 2.5 μM (■) rauwolscine on contractions elicited by (−)-NA (○). All responses are expressed as a percentage of either the maximum response to (−)-NA (a) or the maximum response in the control concentration-response curve (b,c,d) and are the mean of a 4–9 observations in different animals. The vertical lines indicate the s.e.mean.

renal vein or, alternatively, a small difference between arterial and venous α_1-adrenoceptors. Nonetheless, the competitive inhibition (slope of Schild plot not significantly different from unity) produced by corynanthine in preparations where the α-adrenoceptor population is either homogeneous (ear vein and left renal vein) or a mixture (lateral plantaris vein and saphenous vein) of the two subtypes is further testimony of the relatively poor selectivity of this isomer for postjunctional α-adrenoceptor subtypes in the rabbit. This is clearly different from that described for postjunctional α_1-adrenoceptors and prejunctional α_2-adrenoceptors in the rat (Doxey *et al.*, 1984; McGrath, 1984), where both corynanthine and rauwolscine exhibit a similar degree of selectivity for their respective subtype (see: Figure 5). In spite of these species differences, we do not know of any preparation for which the major α-adrenoceptor subtype cannot be characterized by the relative potency of these two antagonists. Indeed, the agreement between antagonist and agonist 'profiles' in the present study, despite the slightly different experimental conditions employed for arterial and venous preparations, further emphasises the utility of these two antagonists and argues against the use of absolute pA_2 values alone in the determination of the major α-adrenoceptor subtype.

Since the isomeric potency ratio of corynanthine and rauwolscine is an expedient indicator of the major subtype present, it may be thought also to reflect the ratio of subtypes present. However, the value of this ratio is limited because of either, (a) significant deviations of the slope of the Schild plot from unity or, (b) the presence of a small resistant component which necessitates determination of the agonist concentration-ratio at a level other than 50% of the maximum response. Thus, in the lateral saphenous vein, a preparation which appears to have both subtypes, the potency of rauwolscine and the isomeric potency ratio are greater than those observed in the ear vein, a preparation which we

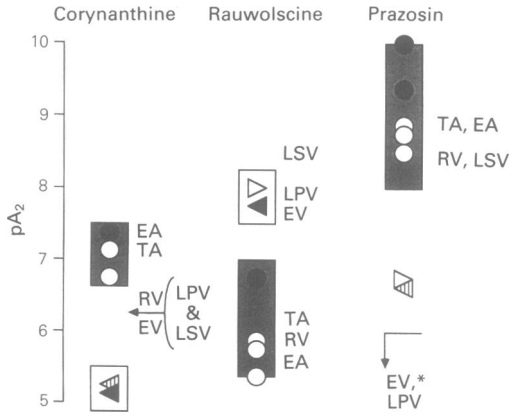

Figure 5 A comparison of the potency of prazosin and the yohimbine diastereoisomers corynanthine and rauwolscine, as antagonists against contractions elicited by noradrenaline (NA) in several isolated blood vessels from the rabbit: thoracic aorta (TA); ear artery (EA); renal vein (RV); lateral plantaris vein (LPV); lateral saphenous vein (LSV); ear vein (EV). The predicted potency range for the antagonists at α_1- and α_2-adrenoceptors are shown by the open and stippled boxes, respectively. This has been based upon reported pA_2 values for the antagonists at post-α_1 (○)-, pre-α_2 (◁)- and post-α_2 (▷)-adrenoceptors in the rat (closed symbols) and rabbit (open symbols). The references for these values can be found in Daly *et al.* (1988b). 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%. Because of the lack of data on postjunctional α_2-adrenoceptors *in vitro* from the rat, comments regarding the selectivity of the antagonists in each species are based upon values determined at *pre*junctional α_2-adrenoceptors. In general, corynanthine shows a greater degree of selectivity for the α_1-adrenoceptor subtype in the rat than in the rabbit. This is emphasized by the wide separation of the closed symbols (rat) compared to the open symbols and data from the present study (rabbit). In marked contrast, rauwolscine appears to possess the opposite spectrum, being more selective for the α_2-subtype in the rabbit than in the rat (NB: the wide separation of the open symbols compared to the closed symbols). The selectivity of prazosin for the α_1-subtype appears to be greater in the rat than in the rabbit, though this is very much dependent upon the assay system adopted (see Table 5, Daly *et al.*, 1988b).

believe to possess almost exclusively the α_2-subtype (Table 2; Figure 5) (Daly *et al.*, 1988a).

The value of prazosin and the selective α-adrenoceptor agonists

The absence of functional postjunctional α_2-adrenoceptors in Group 1 blood vessels, as suggested by the high corynanthine/rauwolscine ratio, is supported by the high potency and competitive antagonism produced by prazosin against NA contractions in the thoracic aorta (Honda *et al.*, 1985), ear artery (Purdy *et al.*, 1980; Hieble *et al.*, 1982) and the left renal vein (Schultz & Westfall, 1982). While the pA_2 values are less than those obtained for prazosin at α_1-adrenoceptors from the rat (see: Figure 5), they are within the expected range for α_1-adrenoceptor antagonist activity in the rabbit. This clearly indicates that although α-adrenoceptor subtypes in different species may possess qualitatively similar characteristics, the selectivity of an antagonist for a particular subtype should, wherever possible, be based upon values obtained from the species in question (see results with corynanthine and rauwolscine as well; Figure 5).

The wide variation in the intrinsic activity values for UK-14304 in the ear artery (0.7), aorta (0.4) and left renal vein (<0.05) may be adequately explained in terms of differences in the receptor reserve for α_1-adrenoceptor-mediated responses. Purdy *et al.* (1983) have demonstrated that only 1% of the receptors in the ear artery have to be activated by NA to produce 50% of the maximum response compared to 16% of the receptor population in the thoracic aorta. The relatively low potency of NA in the renal vein (10 fold less than the aorta and ear artery) suggests that receptor reserve in this preparation is low and is consistent with the poor agonist activity of UK-14304.

The important influence that receptor reserve can exert on the potency of agonists is also evident from a comparison of the potency of phenylephrine in the left renal vein (α_1-) and in the ear vein (α_2-). In spite of the evidence that the two receptors are different, as judged by the effect of corynanthine and rauwolscine on contractions produced against the non-selective agonist NA, phenylephrine was equieffective in each preparation. Confirmation that phenylephrine can mediate responses through α_2-adrenoceptors is provided by the observation that rauwolscine was more potent than corynanthine against phenylephrine-induced contractions in the lateral saphenous vein (Daly *et al.*, 1988b).

Thus, contractile responses elicited by NA in the thoracic aorta, the ear artery and the left renal vein of the rabbit possess the characteristics of the α_1-subtype as described by McGrath (1982) – prazosin-sensitive and corynanthine > rauwolscine.

The absence of a large population of postjunctional α_1-adrenoceptors in the ear vein (Group 2), as suggested by the high potency of rauwolscine, is supported by the failure of prazosin to effect a concentration-dependent inhibition of responses to either NA (present study) or UK-14304 (Daly *et al.*, 1988a). Moreover, the potent and selective α_1-adrenoceptor antagonist YM-12617 (Honda *et al.*,

1985) failed to affect responses to NA in the ear vein (Daly et al., 1988a). Thus, contractile responses elicited by NA in the isolated ear vein of the rabbit possess the primary characteristics of the α_2-subtype as described by McGrath (1982) – prazosin-resistant and rauwolscine > corynanthine. In addition, all three agonists elicited maximum responses of similar magnitude.

Although the presence of postjunctional α_2-adrenoceptors in the lateral saphenous vein and lateral plantaris vein is suggested by the corynanthine: rauwolscine potency ratio against NA contractions (Table 2), evidence for a small population of α_1-adrenoceptors is indicated by a prazosin-sensitive component of the NA CRC in the lateral plantaris vein (Figure 3c) and a rauwolscine-resistant component of responses to NA in the lateral saphenous vein (Figure 4d). Moreover, although UK-14304 was more potent than either NA or phenylephrine in both preparations, the maximum response elicited was significantly smaller than those to these agonists.

It is surprising, therefore, that while there was a prazosin-resistant (rauwolscine-sensitive) component of responses to NA in the lateral plantaris vein (consistent with the characteristics of an α_2-subtype) a similar component was not observed in the lateral saphenous vein (Figure 4c) where the selective α_2-adrenoceptor agonist UK-14304 was just as potent as in the lateral plantaris vein. When examined over a wide concentration range (100 fold) prazosin produced a near parallel rightward displacement of the NA CRC though, as judged by the slope of the Schild plot, the inhibition was non-competitive. The extrapolated pA_2 value for prazosin was 8.44, a value similar to its potency at postjunctional α_1-adrenoceptors (Table 2; Figure 5). This observation is in agreement with those of Purdy et al. (1960) and Schümann & Lues (1983) in this isolated vein. Similarly, although the slope of the Schild plot for rauwolscine against NA in the lateral plantaris vein was not significantly different from unity, there was no evidence of a resistant component (α_1-) as noted in the lateral saphenous vein. Thus, while both preparations appear to possess a mixture of α_1- and α_2-subtypes, the use of a highly selective competitive antagonist is not necessarily guaranteed to reveal the insensitive subtype. A detailed pharmacological examination of the α-adrenoceptors on the lateral saphenous vein is given elsewhere (Daly et al., 1988b), but the factors that result in such a different 'antagonist profile' in two isolated preparations known to possess a mixture of the same α-adrenoceptor subtypes warrant closer examination.

The action of prazosin against NA in five out of six isolated blood vessels was consistent with that expected for a potent and selective α_1-adrenoceptor antagonist. The one exception, however, the lateral saphenous vein, has important consequences for the routine use of prazosin alone for the identification of pharmacological preparations that possess postjunctional α_2-adrenoceptors; no longer can sensitivity to prazosin simply be equated to the absence of postjunctional α_2-adrenoceptors.

In the course of this study we have characterized two new preparations that possess a population of postjunctional α_2-adrenoceptors, the rabbit isolated ear vein and the rabbit isolated plantaris vein. The superficial location of these veins, compared to the renal vein which appears to possess a homogeneous population of postjunctional α_1-adrenoceptors, confirms the view that the α_2-subtype has a major role in thermoregulatory processes (Flavahan & Vanhoutte, 1986), at least in the more peripheral part of the venous system.

Overall, our results indicate that the major postjunctional α-adrenoceptor subtype in any isolated blood vessel from the rabbit can be readily identified by a comparison of the relative potency of the yohimbine diastereoisomers rauwolscine and corynanthine against the 'endogenous' adrenoceptor agonist NA. Furthermore, this relationship may help to overcome the inevitable problems inherent in relying upon the relative potency of two structurally dissimilar ligands (eg; prazosin and yohimbine) for the identification of α_1- and α_2-adrenoceptor subtypes in isolated blood vessels (e.g.: Agrawal et al., 1985) which, as highlighted by Fuder et al., (1981), is critically dependent upon both the physiochemical properties of the ligand and the nature of the receptor biophase. Furthermore, their use may help to reconcile a number of paradoxical observations obtained with selective α_2-adrenoceptor antagonists and prazosin against contractions elicited by selective α_2-adrenoceptor agonists in isolated blood vessels from the rat (see: Weiss et al., 1983; Medgett & Langer, 1984).

With respect to the use of NA, there is accumulating evidence that a number of the synthetic α-adrenoceptor agonists, particularly the imidazolines, initiate contractile responses in vascular smooth muscle that differ in some respects from those elicited by catecholamines (Holck et al., 1983; Chui et al., 1986; Bou & Massingham, 1986) and, indeed, may be capable of evoking responses that cannot be mimicked by catecholamines (Bosquet et al., 1983; Weetman & Coates, 1983). Thus, although selective synthetic agonists are of increasing value in the identification of α-adrenoceptor subtypes (evident by the use of UK-14304 in the present study), confirmation should be attempted with the 'endogenous ligand' NA where possible.

In conclusion, the findings of the present study represent a reassertion of the importance of NA and

the alkaloid diastereoisomers, corynanthine and rauwolscine, as tools for the study of α-adrenoceptor subtypes (Furchgott, 1972; Weitzell *et al.*, 1979; McGrath, 1982).

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References

AGRAWAL, D.K., TRIGGLE, C.R. & DANIEL, E.E. (1985). Pharmacological characterization of the postsynaptic alpha-adrenoceptors in the vascular smooth muscle from canine and rat mesenteric vascular beds. *J. Pharmacol. Exp. Ther.*, **229**, 831–838.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BENTLEY, S.M., DREW, G.M. & WHITING, S.B. (1977). Evidence for two distinct types of postsynaptic α-adrenoceptors. *Br. J. Pharmacol.*, **61**, 116P–117P.

BOSQUET, P., FELDMAN, J. & SCHWARTZ, J. (1983). Central cardiovascular effects of alpha adrenergic drugs: Differences between catecholamine and imidazolines. *J. Pharmacol. Exp. Ther.*, **230**, 232–236.

BOU, J. & MASSINGHAM, R. (1986). Effects of diltiazem upon contractile responses to phenylephrine, cirazoline, Sgd 101/75, St-587 and B-HT 920 in rabbit aorta and dog saphenous vein preparations. *Eur. J. Pharmacol.*, **121**, 319–325.

CAMBRIDGE, D. (1981). UK-14304, a potent and selective $alpha_2$-adrenoceptor agonist for the characterization of alpha-adrenoceptor subtypes. *Eur. J. Pharmacol.*, **72**, 413–415.

CHUI, A.T., McCALL, D.E., THOOLEN, M.J.M.C. & TIMMERMANS, P.B.M.W.M. (1986). Ca^{2+} utilization in the constriction of rat aorta to full and partial $alpha_1$-adrenoceptor agonists. *J. Pharmacol. Exp. Ther.*, **238**, 224–231.

DREW, G.M. & WHITING, S.B. (1979). Evidence for two distinct subtypes of post-synaptic α-adrenoceptors in vascular smooth muscle *in vivo*. *Br. J. Pharmacol.*, **67**, 207–216.

DREW, G.M. (1985). What do antagonists tell us about alpha-adrenoceptors? *Clin. Sci.*, **68**, Suppl. 10, 15s–20s.

DALY, C.J., McGRATH, J.C. & WILSON, V.G. (1988a). Evidence that population of postjunctional adrenoceptors mediating contraction of smooth muscle in the rabbit isolated ear vein is predominantly α_2. *Br. J. Pharmacol.*, **94**, 1085–1090.

DALY, C.J., McGRATH, J.C. & WILSON, V.G. (1988b). 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. *Br. J. Pharmacol.*, **95**, 485–500.

DOCHERTY, J.R., CONSTANTINE, J.W. & STARKE, K. (1981). Smooth muscle of rabbit aorta contains $alpha_1$- but not $alpha_2$-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **317**, 5–7.

DOXEY, J.C., LANE, A.C., ROACH, A.G. & VIRDEE, N.K. (1984). Comparison of the alpha-adrenoceptor antagonist profiles of idazoxan (RX 781094), yohimbine, rauwolscine and corynanthine. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **325**, 136–144.

FLAVAHAN, N.A. & McGRATH, J.C. (1980). Blockade by yohimbine of prazosin-resistant pressor effects of adrenaline in the pithed rat. *Br. J. Pharmacol.*, **69**, 355–358.

FLAVAHAN, N.A., RIMELE, T.J., COOKE, J.P. & VANHOUTTE, P.M. (1984). Characterization of postjunctional $alpha_1$- and $alpha_2$-adrenoceptors activated by exogenous or nerve-released norepinephrine in the canine saphenous vein. *J. Pharmacol. Exp. Ther.*, **230**, 699–705.

FLAVAHAN, N.A. & VANHOUTTE, P.M. (1986). The effect of cooling on $alpha_1$- and $alpha_2$-adrenergic responses in canine saphenous vein and femoral veins. *J. Pharmacol. Exp. Ther.*, **238**, 139–147.

FUDER, H., NELSON, W.L., MILLER, D.D. & PATIL, P.N. (1981). Alpha-adrenoceptors of the rabbit aorta and stomach fundus. *J. Pharmacol. Exp. Ther.*, **217**, 1–9.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology*, Vol XXXIII. ed. Blaschko, H. & Muscholl, E. pp. 283–355, Berlin: Springer-Verlag.

GUIMARAES, S. & PAIVA, M.Q. (1981). Two distinct adrenoceptor-biophases in the vasculature: one for alpha- and the other for β-agonists. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **316**, 195–199.

HIEBLE, J.P., SARAU, H.M., FOLEY, J.J., DEMARINIS, R.M. & PENDLETON, R.G. (1982). Comparison of central and peripheral $alpha_1$-adrenoceptors. *Naunyn-Schmiedebergs Arch Pharmacol.*, **318**, 267–273.

HENSELING, M. (1983). Kinetic constants for uptake and metabolism of ^{3}H-(−)-noradrenaline in rabbit aorta. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **323**, 12–23.

HOLCK, M.I., JONES, C.H.M. & HAUESLER, G. (1983). Differential interactions of clonidine and methoxamine with the postsynaptic alpha-adrenoceptors of rabbit main pulmonary artery. *J. Cardiovasc. Pharmacol.*, **5**, 240–249.

HONDA, K., TAKENAKA, T., MIYATA-OSAWA, A., TERAI, M. & SHIONO, K. (1985). Studies on YM-12617: A selective and potent antagonist of postsynaptic $alpha_1$-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 264–272.

HOOKER, C.S., CALKINS, P.J. & FLEISCH, J. (1976). On the measurement of vascular and respiratory smooth muscle responses *in vitro*. *Blood Vessels*, **14**, 1–11.

JOHNSON, S.M. & DE LA LANDE, I.S. (1978). Action of deoxycorticosterone acetate on the central artery of the rabbit ear. *Blood Vessels*, **15**, 231–246.

McGRATH, J.C. (1982). Evidence for more than one type of post-junctional alpha-adrenoceptor. *Biochem. Pharmacol.*, **31**, 467–484.

McGRATH, J.C. (1984). α-Adrenoceptor antagonism by apoyohimbine and some observations on the pharmacology of α-adrenoceptors in the rat anoccocygens and vas deferens. *Br. J. Pharmacol.*, **82**, 769–781.

MEDGETT, I.C. & LANGER, S.Z. (1984). Heterogeneity of smooth muscle alpha adrenoceptors in rat tail artery *in vitro*. *J. Pharmacol. Exp. Ther.*, **229**, 823–830.

PURDY, R.E., KRUEGER, C.G. & YOUNG, S. (1980). Evidence for non-classical alpha-adrenoceptor blockade by prazosin in isolated rabbit blood vessels. *Life Sci.*, **27**, 2187–2195.

PURDY, R.E., ASHBROOK, D.W., STUPECKY, G.L. & WATANABE, M.Y. (1983). Qualitative and quantitative differences between the postsynaptic alpha-adrenoceptors of the rabbit ear artery and the thoracic aorta. *J. Pharmacol. Exp. Ther.*, **224**, 543–551.

RUFFOLO, R.R. JR., ROSING, E.L. & WADDELL, J.E. (1979). Receptor interactions of imidazolidines. I. Affinity and efficacy for alpha-adrenergic receptors in rat aorta. *J. Pharmacol. Exp. Ther.*, **209**, 429–436.

SCHULTZ, J.C. & WESTFALL, D.P. (1982). A pharmacological analysis of the alpha-adrenoceptor antagonism by prazosin in arteries and veins. *Blood Vessels*, **19**, 79–87.

SCHÜMANN, H-J. & LUES, I. (1983). Postjunctional alpha-adrenoceptors in the isolated saphenous vein of the rabbit. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **323**, 328-334.

SHOJI, T., TSURU, H. & SHIGEI, T. (1983). A regional difference in the distribution of postsynaptic alpha-adrenoceptor subtypes in canine veins. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **324**, 246–255.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn. Ther.*, **143**, 229–330.

WEETMAN, D.F. & COATES, J. (1983). Occurrence of α_{1_B}-adrenoceptors in the mouse but not in the rabbit isolated anococcygeus preparations. *Br. J. Pharmacol.*, **78**, 117–123.

WEISS, R.J., WEBB, R.C. & SMITH, C.B. (1983). Alpha$_2$-adrenoceptors on arterial smooth muscle: selective labelling by [^{3}H] clonidine. *J. Pharmacol. Exp. Ther.*, **225**, 599–605.

WEITZELL, R., TANAKA, T. & STARKE, K. (1979). Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn Schmiedebergs Arch. Pharmacol.*, **308**, 127–136.

WILFFERT, B., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1982). Extrasynaptic location of α_2- and noninnervated β_2-adrenoceptors in the vascular system of the pithed normotensive rat. *J. Pharmacol. Exp. Ther.*, **221**, 762–768.

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