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Evidence that the population of postjunctional-adrenoceptors mediating contraction of smooth muscle in the rabbit isolated ear vein is predominantly α_2

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1 Noradrenaline (NA), phenylephrine and UK-14304 elicited concentration-dependent contractions of the rabbit isolated ear vein of similar maximal magnitude. The rank order of potency, UK-14304 > noradrenaline > phenylephrine, is consistent with that of an effect mediated through an α_2-subtype.

2 The potent and highly selective α_1-adrenoceptor antagonists prazosin and YM-12617, at concentrations as high as 1 μM, produced less than a 4 fold rightward displacement of the NA concentration-response curve.

3 The selective α_2-adrenoceptor antagonists rauwolscine, Wy-26703 and CH-38083 antagonized responses to noradrenaline in a competitive manner. For all three antagonists, the pA_2 values were consistent with an effect at α_2-adrenoceptors. However, 0.1 μM YM-12617 increased the potency of rauwolscine 2 fold indicating the presence of a small population of postjunctional α_1-adrenoceptors.

4 The relative antagonist potency of the yohimbine diastereoisomers rauwolscine and corynanthine against noradrenaline (rauwolscine 30 fold > corynanthine) is also consistent with an effect at α_2-adrenoceptors.

5 Contractions elicited by noradrenaline in the rabbit isolated ear vein appear to be mediated predominantly by postjunctional α_2-adrenoceptors.

Introduction

Postjunctional α-adrenoceptors in the vasculature have been subdivided into α_1- and α_2-subtypes mainly upon evidence obtained from studies with 'selective' agonists and antagonists in conscious and pithed animals (McGrath, 1982). While there is abundant evidence for postjunctional α_1-adrenoceptors in isolated blood vessels, there are very few isolated preparations that appear to possess the α_2-subtype (eg: the dog isolated saphenous vein – Constantine *et al.*, 1982; the rat isolated saphenous vein – Cheung, 1985; the rabbit isolated saphenous vein – Schümann & Lues, 1983; the rat isolated femoral vein – Downing *et al.*, 1986). The α-adrenoceptor population in most of these preparations, however, represents a mixture of both subtypes so examination of either the physiological or pharmacological characteristics of contractions mediated by the α_2-subtype necessitates the use of either 'selective' agonists (non-phenethylamines) or selective antagonists, or both (see: Flavahan & Vanhoutte, 1986).

A number of recent observations suggest that while non-phenethylamine agonists (e.g.: imidazolines) are particularly useful in identifying subtypes of α-adrenoceptors, the functional response arising from stimulation of the receptor may differ qualitatively from that produced by phenylethylamines. Langer & Dubocovich (1981) and Hicks *et al.* (1985) have noted that inhibition of neuronal uptake has qualitatively different effects on the prejunctional activity of imidazolines and phenylethylamines at α_2-adrenoceptors, while Bou & Massingham (1986)

and McGrath (1983) have shown that the ability of various 'selective' agonists to mobilize cellular calcium via postjunctional α_1- or α_2-adrenoceptors does not correlate with either their selectivity for, or efficacy at, a particular subtype, but, rather, appears to be a function of the chemical structure of the agonist. Moreover, one of the 'selective' α_2-adrenoceptor agonists used in many studies, B-HT-920, has been shown to possess agonist activity at dopamine receptors (de Jonge *et al.*, 1984; Hinsen *et al.*, 1986). Thus, if we are to gain further insight into the physiological function of vascular postjunctional α_2-adrenoceptors, an isolated preparation that permits the examination of receptor function with the phenylethylamine agonists is required.

Our observations with the rabbit isolated ear vein suggest that it contains a large population of α_2-adrenoceptors and, as such, could be useful in the examination of the characteristics of this subtype with the 'endogenous' ligand (−)-noradrenaline (NA).

Methods

White albino New Zealand rabbits (2.3–3.2 kg) of either sex were killed by stunning followed by exsanguination. Two 4–5 mm length segments of the ear vein were carefully dissected from each ear and placed in ice-cold physiological salt solution (PSS). Two 0.2 mm thick stainless steel wires were placed in the lumen of the vessels as described by Hooker *et al.* (1976) and each segment was suspended under a resting tension of 75–100 mg in a 30 ml isolated organ bath containing physiological salt solution (PSS) maintained at 37°C and gassed with $95\%O_2 : 5\%CO_2$. Contractions were measured by a Grass FT03 isometric force transducer and displayed on a Linseis 2065 chart recorder.

Following an equilibration period of 60 min, preparations were exposed to 3 μM NA for 5 min and then repeatedly washed with PSS to effect complete relaxation. Sixty min later cumulative concentration-response curves to NA, phenylephrine and UK-14304 were then constructed and the potency of an agonist was expressed as the negative logarithm of the concentration that caused 50% of maximum contraction (pD_2—Van Rossum, 1963). For NA (all antagonists) and UK-14304 (prazosin only) concentration-response curves were repeated after 45 min equilibration with a maximum of two concentrations of an antagonist (a maximum of 3 concentration-response curves per preparation). Agonist concentration-ratios produced by the antagonists were determined from the rightward displacement of the concentration-response curve at the 50% of maximum (control) response, and pA_2 values determined using regression analysis from the pooled data by the method of Arunlakshana & Schild (1959) from a minimum of three concentrations.

The composition of the PSS was (in mM) NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 11 and Na_2EDTA 0.023. Propranolol 1 μM and cocaine 10 μM were also included to inhibit β-adrenoceptors and $Uptake_1$, respectively. The following compounds were used: prazosin HCl (Pfizer); corynanthine HCl (Roth); rauwolscine HCl (Roth); Wy-26703 (N-methyl-N-(1,3,4,6,7,11b-hexahydro-2H-benzo-[a]-quinolizin-2-yl)-i-butanesulphonamide HCl, Wyeth); YM-12617 (5-[2-[[2-(ethoxyphenoxy)ethyl]amino]propyl]-2-methoxy benzene-sulphonamide HCl, Yamanouchi); CH-38083 (7,8-(methylenedioxi)-14-α-hydro-alloberbane HCl, Chinoin); UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate, Pfizer); phenylephrine HCl (Sigma); noradrenaline bitartrate (Sigma); propranolol HCl (Sigma) and cocaine HCl (MacCarthys).

Differences between mean values were tested by Student's t test and were considered statistically significant if $P < 0.05$ or if the 95% confidence limits overlapped.

Results

Agonist-induced responses

NA elicited concentration-dependent contractions of the rabbit isolated ear vein characterized by a peak response attained within 2–3 min of drug addition followed by a slow relaxation (Figure 1). In a number of preparations, small phasic contractions were superimposed upon the sustained response. Both UK-14304 and phenylephrine produced responses of similar time course. All subsequent cumulative additions of the agonist were made as close as possible to the peak of the preceding response. Successive concentration-response curves

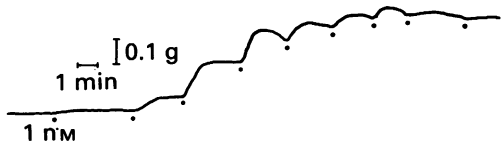

Figure 1 Representative trace recording of contractions of the rabbit isolated ear vein to the cumulative addition of noradrenaline (NA). (●) Denotes the approximately 3 fold increase in the concentration of NA.

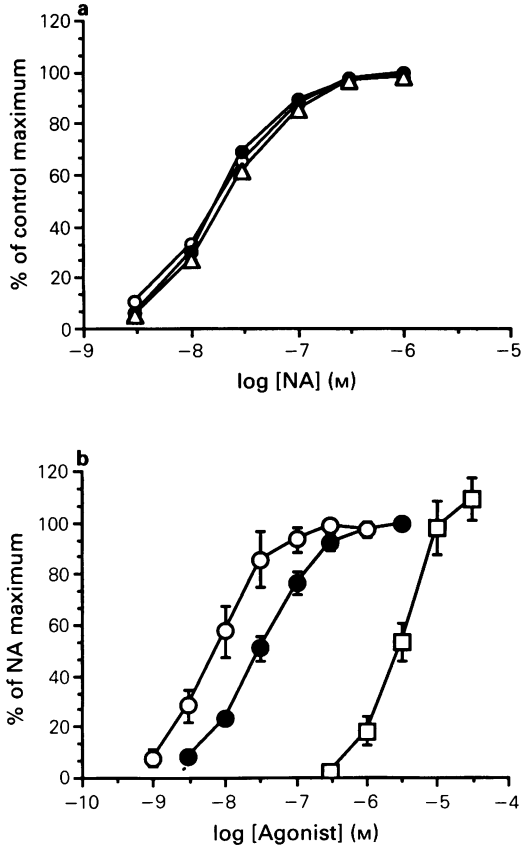

Figure 2 (a) A comparison of three successive cumulative concentration-response curves to noradrenaline (NA) in the rabbit isolated ear vein: 1st (●), 2nd (○) and 3rd (△). All responses have been expressed as a percentage of the maximum response to NA of its own concentration-response curve ($n = 7$). The s.e.mean of responses (<6%) have not been included. (b) A comparison of the effects of UK-14304 (○), NA (●) and phenylephrine (□) in the rabbit isolated ear vein. Responses have been expressed as a percentage of the maximum response to NA. Each point represents the mean of 5 observations and the vertical lines indicate the s.e.mean.

to NA showed a significant increase in the maximum response in the 2nd (13.5 ± 3.55%; $n = 7$) and 3rd (13.0 ± 4.5%; $n = 7$) curves, but a comparison of the curves based upon their own maximum revealed that there was no significant change in the sensitivity of the preparation (Figure 2a). In view of the small changes in the maximum response and sensitivity of the preparation, no attempt has been made to correct for a time-dependent shift in calculating the agonist concentration-ratio values produced by antagonists.

Maximum contractions to all three agonists (NA: 397 ± 70 mg, phenylephrine: 434 ± 65 mg, UK-14304: 386 ± 50 mg; $n = 5$) were not significantly different, but UK-14304 (pD_2—8.11 ± 0.16, $n = 5$) was 3.7 fold more potent than NA (pD_2—7.53 ± 0.08, $n = 5$) and 370 fold more potent than phenylephrine (pD_2—5.54 ± 0.06, $n = 5$, see Figure 2b).

The effects of antagonists on NA-induced contractions

Prazosin 0.1 μM caused a 2 fold rightward displacement of the NA concentration-response curve ($n = 9$), but a 10 fold increase in the concentration of the antagonist failed to effect a greater inhibition (Figure 3a). Similarly, 0.01 μM YM-12617 did not affect responses to NA, while 0.1 μM and 1 μM caused less than a 4 fold displacement of the NA concentration-response curve (Figure 3b).

In marked contrast, Wy-26703 (Figure 3c), CH-38083 (Figure 3d), rauwolscine and corynanthine caused a concentration-dependent, parallel, rightward displacement of the concentration-response curve for (−)-NA. For each antagonist, the 95% confidence interval for the slope of the Schild plot encompassed unity and, therefore, the slope was not significantly different from unity (Table 1). Based upon the 95% confidence limits for the pA_2 values, the rank order of potency for the antagonists was: rauwolscine = CH - 38083 = Wy - 26703 > corynanthine. With the exceptions of the highest concentrations of rauwolscine (10 μM) and CH-38083 (10 μM),

Table 1 pA_2 values and the slope of the Schild plot (with 95% confidence limits) for the effect of several competitive antagonists against noradrenaline in the rabbit isolated ear vein

Antagonist	*pA_2*	*Slope*
Rauwolscine	7.7	1.08
(0.05 μM–2.5 μM)	(7.94–7.45)	(0.91–1.26)
Rauwolscine (YM-12617)*	8.04	1.03
(0.05 μM–2.5 μM)	(8.34–7.65)	(0.85–1.22)
CH-38083	7.77	0.94
(0.1 μM–10 μM)	(8.01–7.55)	(0.83–1.05)
Wy-26703	7.35	0.91
(0.1 μM–10 μM)	(7.58–7.11)	(0.78–1.04)
Corynanthine	6.22	0.92
(2.5 μM–50 μM)	(6.45–5.98)	(0.76–1.08)

Values shown are from a minimum of 14 determinations at three different concentrations.
* In the presence of 0.1 μM YM-12617.

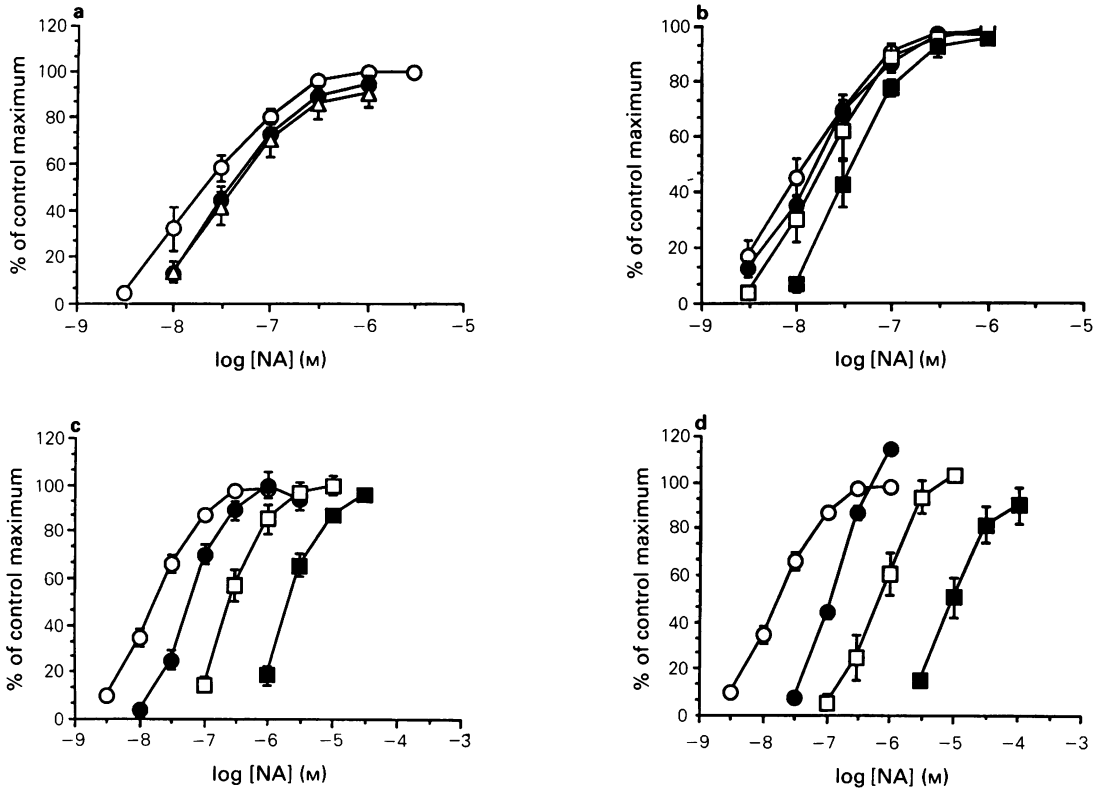

Figure 3 The effects of various antagonists on responses to noradrenaline (NA) in the rabbit isolated ear vein: (a) prazosin 0.1 μM (●) and 1 μM (△); (b) YM-12617 0.01 μM (●), 0.1 μM (□) and 1 μM (■); (c) Wy-26703 0.1 μM (●), 1 μM (□) and 10 μM (■); (d) CH-38083 0.1 μM (●), 1 μM (□) and 10 μM (■). Control concentration-response curves to NA are represented by (○) on all graphs. Each point represents the mean of 4–9 observations and the vertical lines indicate the s.e.mean.

none of the antagonists caused a significant reduction in the maximum response elicited by NA, and even in these two instances, it is possible that with a higher concentration of NA the control maximum might have been attained.

In the presence of 0.1 μM YM-12617, which alone produced less than a 2 fold rightward displacement of the NA concentration-response curve, the pA_2 value for rauwolscine was increased from 7.7 to 8.0 (Table 1). However, since the 95% confidence limits for both values overlapped this increase did not reach significance.

The effects of prazosin on contractions elicited by UK-14304

Prazosin, 0.1 μM and 1 μM, failed to affect responses elicited by UK-14304 (Figure 4).

Discussion

McGrath (1982) defined responses mediated by post-junctional α_2-adrenoceptors as those which are insensitive to the selective α_1-adrenoceptor antagonist prazosin, but which are more sensitive to the selective α_2-adrenoceptor antagonist rauwolscine than to its diastereoisomer corynanthine, the latter being relatively more potent at α_1-adrenoceptors. Contractions to NA in the rabbit isolated ear vein appear to possess these characteristics. Firstly, responses to NA were relatively insensitive to 0.1 μM and 1 μM prazosin (concentrations that would cause a 50 fold and 500 fold shift, respectively, of the NA concentration-response curve at α_1-adrenoceptors in the rabbit isolated thoracic aorta, pA_2—8.7; Docherty *et al.*, 1981) and, secondly, rauwolscine was 30 fold more potent than corynanthine. Furthermore, the pA_2 value for rauwolscine (7.7) against NA-induced contractions agrees well with that

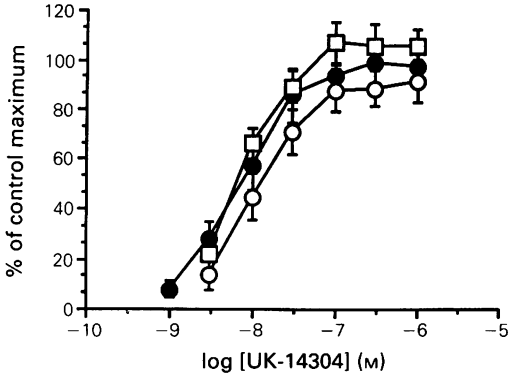

Figure 4 The effects of 0.1 μM (○) and 1 μM (□) prazosin on contractions produced by UK-14304 (●) in the rabbit isolated ear vein. Each point represents the mean of 5–6 observations and the vertical lines indicate the s.e.mean.

observed at postjunctional α_2-adrenoceptors in the rabbit isolated saphenous vein (Alabaster *et al.*, 1985). Thus, the adrenoceptors mediating contraction in this preparation appear to be of the α_2-subtype.

This view is supported by a number of other observations with selective antagonists. YM-12617 is a potent antagonist which possesses selectivity for α_1-adrenoceptors in the rat comparable to that of prazosin (Honda *et al.*, 1985; McGrath & Wilson, 1987), and in the rabbit greater than that of prazosin (Honda *et al.*, 1985; Takayanagi *et al.*, 1986). Based upon a pA_2 value of 10 for YM-12617 at postjunctional α_1-adrenoceptors, 0.01 μM would be expected to cause a 100 fold rightward displacement of the concentration-response curve to NA produced via α_1-adrenoceptors, but in the rabbit isolated ear vein this concentration was inactive. Furthermore, the 4 fold rightward displacement produced by 1 μM YM-12617 is consistent with an effect at α_2-adrenoceptors (pA_2 of 6.5 at prejunctional α_2-adrenoceptors on the rat vas deferens; Honda *et al.*, 1985). The selective α_2-adrenoceptor antagonists Wy-26703 (Lattimer *et al.*, 1984) and CH-38083 (Vizi *et al.*, 1986) both produced competitive antagonism of responses to NA with a potency consistent with an effect at an α_2-subtype.

The rank order of agonist potency in this preparation also suggests the presence of a large population of postjunctional α_2-adrenoceptors. The selective α_2-adrenoceptor agonist UK-14304 (Cambridge 1981) was approximately 4 fold more potent than NA and 400 fold more potent than the selective α_1-adrenoceptor agonist phenylephrine (McGrath 1982), but all three agonists elicited comparable maximal responses. The similar intrinsic activity of these agonists in the ear vein is suggestive of a homogeneous population of α-adrenoceptors, and stands in marked contrast to other preparations demonstrated to possess a population of postjunctional α_2-adrenoceptors where, invariably, the maximum responses to the selective α_2-adrenoceptor agonists are smaller than those to either NA or phenylephrine (see: Schümann & Lues, 1983; Alabaster *et al.*, 1985; Flavahan & Vanhoutte, 1986; Downing *et al.*, 1986). However, the presence of a small population of postjunctional α_1-adrenoceptors is suggested by two observations. First, the small but non-significant increase in the pA_2 value for rauwolscine against NA in the presence of 0.1 μM YM-12617 and, secondly, the significant inhibition produced by 0.1 μM and 1 μM prazosin against NA contractions; an effect not observed against the selective α_2-adrenoceptor agonist UK-14304.

In conclusion, contractile responses elicited by NA in the rabbit isolated ear vein appear to be mediated predominantly by a population of postjunctional α_2-adrenoceptors. The absence of a major contribution by postjunctional α_1-adrenoceptors would appear to make this a suitable preparation for the examination of the functional characteristics of postjunctional vascular α_2-adrenoceptors, since this can be achieved with the non-selective 'endogenous' agonist NA.

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