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Characterization of postsynaptic α-adrenoceptors in rat aortic strips and portal veins

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1 Postsynaptic α-adrenoceptors in rat isolated aortic strips and portal veins have been examined using a number of agonist and antagonist drugs which have varying selectivity for α_1- and α_2-adrenoceptors.

2 In both tissues (−)-noradrenaline ((−)-NA), (−)-adrenaline ((−) Adr) (−)-α-methyl noradrenaline ((−)-α-Me-NA) and (−)-phenylephrine ((−)-PE) were full agonists, while clonidine, oxymetazoline and (2-(2,6-dichlorophenyl)-5,6-dihydroimidazo(2,1,b) thiazole (44,549) were partial agonists. Guanfacine was a full agonist in aortic strips but only a partial agonist in portal veins.

3 In aortic strips, pA_2 values for prazosin and yohimbine were not significantly different using (−)-NA, (−)-PE or guanfacine as the agonist, suggesting a single population of α-adrenoceptors. The order of potency of the antagonists, prazosin = 2-(β-(4-hydroxyphenyl)-ethylaminomethyl)-tetralone (BE2254) > phentolamine > yohimbine > rauwolscine, is indicative of an α_1-type of receptor.

4 In portal veins, the order of potency of the antagonists was prazosin > BE2254 > phentolamine > yohimbine > rauwolscine, again indicating an α_1-type of receptor.

5 The mean pA_2 value for yohimbine was not significantly different in either tissue. However, mean pA_2 values for prazosin, BE-2254 and phentolamine were approximately one order of magnitude lower in portal veins than in aortic strips, suggesting that the receptors in the two tissues may not be identical.

Introduction

There is now considerable evidence to suggest that α-adrenoceptors are not homogeneous, but may be divided into two subtypes, termed α_1 and α_2 (for review see Starke, 1981). Although the original subclassification of adrenoceptors was made in two anatomical areas, i.e. presynaptic receptors = α_2 and postsynaptic receptors = α_1 (Langer, 1974), the receptors are now usually defined by the relative affinities and potencies of agonist and antagonist drugs. Thus, a receptor is said to be α_1 if the relative affinity of (−)-phenylephrine (PE) is greater than clonidine and xylazine, and if the order of potency of antagonists is prazosin ➤ corynanthine > yohimbine > rauwolscine, and α_2 if the relative affinity of clonidine and xylazine is greater than PE and if the order of potency of antagonists is rauwolscine, yohimbine > corynanthine > prazosin (Wikberg, 1978; 1979; Starke 1981).

The existence of postsynaptic α_2-adrenoceptors in vascular smooth muscle has been suggested by a number of *in vivo* experiments in pithed rat (Bentley, Drew & Whiting, 1977; Drew & Whiting, 1979; Timmermans, Kwa & Van Zweiten, 1979; Docherty, MacDonald & McGrath, 1979; Docherty & McGrath, 1980a,b), in anaesthetized cat (Drew & Whiting, 1979), conscious rabbit (Hamilton & Reid, 1980) and in the autoperfused hindlimb of the dog (Langer, Massingham & Shepperson, 1981a,b) and rabbit (Madjar, Docherty & Starke, 1980). (For review on postsynaptic α_2-adrenoceptors, see Timmermans & Van Zweiten, 1981). However, there is much less evidence from experiments performed on isolated blood vessels *in vitro* to suggest that α_2-adrenoceptors exist postsynaptically. Human isolated digital arteries have been shown to possess adrenoceptors which are resistant to prazosin (Moulds & Jauernig, 1977; Jauernig, Moulds & Shaw, 1978), and which may be of the α_2 type. α_2-Adrenoceptors have also been reported in canine isolated saphenous veins (De Mey & Vanhoutte, 1980; 1981; Shepperson & Langer, 1981), although Sullivan & Drew (1980) concluded that the α-

adrenoceptors in the same tissue were of the α_1-type. In rabbit isolated aorta, pulmonary artery and portal vein, no evidence for the existence of postsynaptic α_2-adrenoceptors could be found (Docherty, Constantine & Starke, 1981; Docherty & Starke, 1981).

It has been suggested, however, that the receptors in rat aorta have some α_2-properties, since they possess a high affinity for clonidine (Ruffolo, Yaden & Waddell, 1980) and in a recent communication, it was postulated that rat aorta contains both α_1- and α_2-adrenoceptors (Godfraind & Miller, 1982). In both these studies the evidence for the existence of α_2-adrenoceptors relies on the relatively high affinity of oxymetazoline and/or clonidine for α_2-receptors.

In the present investigation, a number of agonist and antagonist drugs with varying selectivity for α_1- and α_2-adrenoceptors, have been used to characterize the adrenoceptors in rat isolated aortic strips and portal veins. A preliminary account of some of the present results, has been presented (Digges & Summers, 1982).

Methods

Sprague-Dawley rats (180–240 g) of either sex were killed by a blow on the head. Helical strips of descending thoracic aorta approximately 3 mm × 30 mm (Furchgott & Bhadrakom, 1953) and whole portal veins (20 mm in length) were mounted in 20 ml organ baths containing Krebs-Henseleit solution (composition in mM: NaCl 118.07, KCl 5.36, $MgSO_4.7H_2O$ 0.57, $CaCl_2$ 1.90, $NaH_2PO_4.2H_2O$ 0.90, $NaHCO_3$ 25.0 and glucose 11.1), aerated with 5% CO_2 in O_2, and maintained at 37°C. The Krebs-Henseleit solution contained propranolol (10^{-6} M), desmethylimipramine (10^{-6} M) and normetanephrine (10^{-6} M) to inhibit β-adrenoceptors, neuronal and extraneuronal uptake respectively. The tissues were suspended under either 1 g tension (aortic strips) or 0.5 g tension (portal veins), and responses were recorded via Narco Bio-systems F60 isometric transducers, and displayed on a Narco Bio-systems physiograph recorder. The aortic strips were allowed to equilibrate for at least 90 min, and the portal veins for at least 60 min, before addition of any drugs.

Cumulative concentration-response curves to the agonists were obtained by approximately doubling drug concentrations with each addition. The concentration of agonist was increased only after the previous concentrations had produced its peak response. In each preparation, two control concentration-response curves to noradrenaline (NA) were constructed, and all subsequent results were compared with the second of these curves. A period of 30 min was allowed between each agonist concentration-response curve. Responses were expressed as a percentage of the maximum contractile response to (−)-NA, and EC_{50} values were determined from individual concentration-response curves by regression analysis (over the range 20–80% of the maximum response). The maximum responses of the agonists were used to estimate their intrinsic activities, relative to (−)-NA.

Since EC_{50} values may not be a reliable measure of the actual affinities of agonists for the receptor, dissociation constants of the agonists were calculated using the irreversible antagonist phenoxybenzamine (Pbz) to produce a reduction in the number of available receptors (see Furchgott, 1966; Furchgott & Bursztyn, 1967). In these experiments, a concentration of Pbz (5×10^{-8} M) was added to the organ baths 30 min after the second concentration-response curve to the agonist, and allowed to act for 10 min. The tissues were repeatedly washed over a period of 30 min, before a further concentration-response curve to the agonist was constructed. The dissociation constant of the agonist was calculated from the equation:

$$\frac{1}{[A]} = \frac{1-q}{qK_A} + \frac{1}{q} \cdot \frac{1}{[A']},$$

where [A] and [A′] equal the concentration of agonist A before and after irreversible inactivation of a fraction of receptors with Pbz, respectively; q equals the remaining fraction of active receptors after Pbz, and K_A equals the dissociation constant of agonist A. A plot of 1/[A] against 1/[A′] (i.e. reciprocals of equiactive concentrations of the agonist before and after Pbz) should yield a straight line, and K_A is equal to the ratio of (slope − 1) to intercept (see Furchgott, 1966; Furchgott & Bursztyn, 1967).

EC_{50} values for the partial agonists were determined using the same method as for full agonists (above). Dissociation constants of the partial agonists were calculated from the equation:

$$\frac{1}{[A]} = \frac{e_A}{K_A e_P} + \frac{e_A}{e_P} \cdot \frac{K_P}{K_A} \cdot \frac{1}{[P]},$$

where [A] and [P] equal the concentrations of full agonist (i.e. NA) and partial agonist, respectively; K_A and K_P equal the dissociation constants of the full agonist A and partial agonist P; and e_A and e_P equal the efficacies of A and P respectively (see Waud, 1969; Jenkinson, 1979). A plot of reciprocals of equiactive concentrations of A and P should yield a straight line and K_P is equal to the ratio of the slope to intercept.

In experiments examining the effects of antagonists, a concentration of antagonist was added to the organ baths 30 min after the second concentration-response curve to the agonist, and allowed to equilibrate for 30 min before a further concentration-

response curve was constructed. Three different concentrations of antagonist were examined in each preparation. pA_2 values for the antagonists were calculated from Arunlakshana & Schild (1959) plots, constructed of log (dose-ratio − 1) against log (antagonist concentration). Dose-ratios were calculated at the EC_{50} level.

pK_B values were calculated from the equation

$$K_B = \frac{\text{(concentration antagonist)}}{\text{(dose-ratio} - 1)}$$

for each concentration of antagonist tested (Furchgott, 1972).

Results given are mean±s.e.mean. The following drugs were used: (−)-adrenaline bitartrate (Sigma); BE2254 (2-(β-(4-hydroxyphenyl)-ethylaminomethyl)-tetralone) (Beiersdorf); clonidine hydrochloride (Boehringer); desmethylimipramine hydrochloride (Ciba-Geigy); 44,549 (2-(2,6-dichlorophenyl)-5,6-dihydroimidazo (2,1,b) thiazole (Sandoz); guanfacine hydrochloride (Sandoz); (−)-α-methyl noradrenaline (Sterling-Winthrop); (−)-noradrenaline bitartrate (Sigma); (±)-normetanephrine hydrochloride (Sigma); oxymetazoline hydrochloride (Allen and Hanburys); phenoxybenzamine hydrochloride (Smith, Kline and French); (−)-phenylephrine hydrochloride (Koch-Light); phentolamine hydrochloride (Ciba-Geigy); prazosin hydrochloride (Pfizer); propranolol hydrochloride (Sigma); rauwolscine hydrochloride (Roth) and yohimbine hydrochloride (Baird Pharmaceuticals).

Results

Responses to agonists and partial agonists in rat isolated aortic strips and portal veins

Both aortic strips and portal veins responded to α-adrenoceptor agonists with dose-dependent contractions. Responses of the aortic strips consisted of slow sustained contractions, whereas portal veins all exhibited spontaneous activity which increased in height and sometimes frequency at low concentrations of agonists. In portal veins high concentrations of agonists increased the base-line tension producing a maintained contracture. In all cases responses were measured from the midpoints of the spontaneous contractions. Examples of cumulative dose-response curves to (−)-NA for both tissues are shown in Figure 1a and b.

In aortic strips (−)-NA (−)-adrenaline ((−)-Adr, non-selective) (−)-PE (preferential α_1) and α-methyl noradrenaline ((−)-α-Me-NA) and guanfacine (preferential α_2) were all full agonists (Figure 2a). The maximum tension produced by (−)-NA in these preparations was 0.58 ± 0.03 g ($n = 62$). The α_2 selective compounds clonidine, oxymetazoline and 44,549 were partial agonists in this preparation (Figure 2b). In isolated portal veins (−)-NA (−)-Adr (−)-PE and (−)-α-Me-NA were all full agonists (Figure 3a), but all were significantly less potent than in aortic strips. Guanfacine, which was a full agonist in aortic strips was a partial agonist in isolated portal veins, as were clonidine, oxymetazoline and 44,549

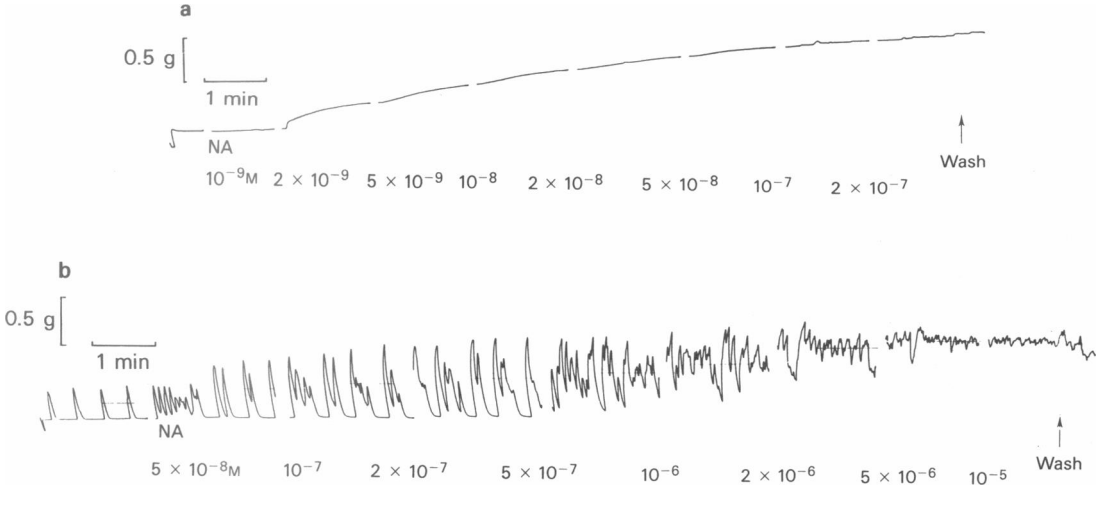

Figure 1 Responses to cumulative addition of (−)-noradrenaline ((−)-NA) to rat isolated aortic strips (a) and portal veins (b) in typical experiments. Isometric tension (g) is recorded on the abcissa scale and time on the ordinate scale together with the cumulative concentration of agonist.

(Figure 3b). The maximum tension in isolated portal veins produced by (−)-NA was 0.74 ± 0.04 g ($n = 62$). Mean EC_{50} values, pD_2s (−log EC_{50}s) and intrinsic activities (relative to (−)-NA) are shown in Table 1 for all agonists and partial agonists in both preparations.

The dissociation constants for (−)-NA, (−)-PE and guanfacine were determined in aortic strips using the antagonist Pbz to block irreversibly a proportion of the α-adrenoceptors. The effects of Pbz on concentration-response curves to each of these three agonists, and plots of reciprocals of equiactive concentrations of the agonists before and after Pbz are shown in Figure 4. The Ka values calculated for (−)-NA, (−)-PE and guanfacine were 11–29 times their respective EC_{50} values (Table 2). The affinity of the partial agonists for the α-adrenoceptor in aortic strips and portal veins was calculated from double reciprocal plots of equiactive concentrations of a full agonist ((−)-NA) and each partial agonist. Typical double reciprocal plots for oxymetazoline and 44,549 are shown for aortic strips in Figure 5 and for isolated portal veins in Figure 5b. Dissociation constants for the partial agonists, unlike the full agonists, were much closer to their pD_2 values (Table 2).

Effects of antagonists on responses to agonists in rat isolated aortic strips and portal veins

In isolated aortic strips and portal veins the antagonists prazosin, BE 2254 (preferential α_1), phentolamine (non selective) and yohimbine and rauwolscine (preferential α_2) all inhibited the agonist activity of (−)-NA, producing parallel shifts to the right of the concentration-response curves without affecting the maximum response. Schild plots from typical experiments for each antagonist are shown for aortic strips in Figure 6a and for portal veins in Figure 6b.

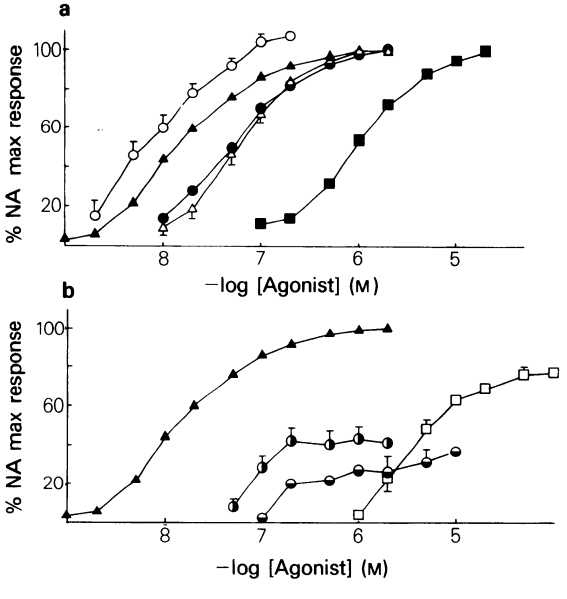

Figure 2 Concentration-response curves for full agonists (a) and partial agonists (b) in isolated aortic strips. (▲) (−)-noradrenaline ((−)-NA) ($n = 62$); (○) (−)-adrenaline ($n = 7$); (△) (−)-α-methyl-NA ($n = 5$); (●) (−)-phenylephrine ($n = 19$); (■) guanfacine ($n = 6$); (□) oxymetazoline ($n = 5$); (○) clonidine ($n = 4$); and (○) 44,549 ($n = 5$). Responses are expressed on the abcissa scale as a percentage of the maximum response to (−)-NA (vertical lines show s.e.mean) and plotted against concentrations of drug (M) on the ordinate scale.

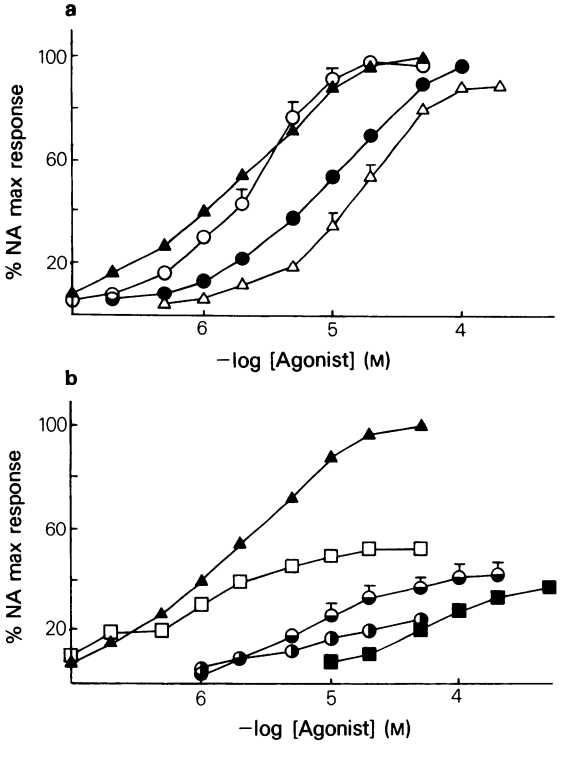

Figure 3 Concentration-response curves for full agonists (a), and partial agonists (b), in rat isolated portal veins. (▲) (−)-noradrenaline ((−)-NA) ($n = 62$); (○) (−)-adrenaline ($n = 7$); (△) (−)-α-methyl-NA ($n = 5$); (●) (−)-phenylephrine ($n = 19$); (■) guanfacine ($n = 6$); (□) oxymetazoline ($n = 5$); (○) clonidine ($n = 4$) and (○) 44,549 ($n = 5$). Responses are expressed on abcissa scale as a percentage of the maximum response to (−)-NA (vertical lines show s.e.mean) and are plotted against concentrations of drug (M) on the ordinate scale.

Table 1 EC_{50} values and intrinsic activity (i.a.) for adrenoceptor agonists and partial agonists in rat isolated aortic strips and portal veins

Agonist	n	Mean EC_{50} (M)	Mean pD_2	i.a.
		Aortic strips		
Noradrenaline	62	$1.49 \pm 0.10 \times 10^{-8}$	7.88 ± 0.03	1
Adrenaline	7	$9.54 \pm 2.28 \times 10^{-9}$	8.09 ± 0.10	1.07
α-Me-NA	5	$6.10 \pm 1.08 \times 10^{-8}$	7.23 ± 0.08	1.01
Phenylephrine	25	$5.59 \pm 0.51 \times 10^{-8}$	7.41 ± 0.05	1.07
Guanfacine	22	$9.36 \pm 0.96 \times 10^{-7}$	6.08 ± 0.05	1.09
Oxymetazoline	5	$3.58 \pm 0.72 \times 10^{-6}$	5.48 ± 0.09	0.78
Clonidine	4	2×10^{-7}*	6.70	0.31
44,549	5	$5.25 \pm 1.47 \times 10^{-7}$	6.39 ± 0.18	0.50
		Portal veins		
Noradrenaline	62	$1.87 \pm 0.11 \times 10^{-6}$	5.78 ± 0.03	1
Adrenaline	7	$2.32 \pm 0.34 \times 10^{-6}$	5.66 ± 0.06	0.99
α-Me-NA	5	$1.69 \pm 0.21 \times 10^{-5}$	4.79 ± 0.6	0.89
Phenylephrine	19	$9.00 \pm 1.15 \times 10^{-6}$	5.12 ± 0.07	0.92
Guanfacine	6	4×10^{-5}*	4.40	0.36
Oxymetazoline	5	$2.25 \pm 0.78 \times 10^{-6}$	5.76 ± 0.15	0.51
Clonidine	4	$6.67 \pm 0.74 \times 10^{-6}$	5.18 ± 0.05	0.46
44,549	5	$4.34 \pm 0.69 \times 10^{-6}$	5.38 ± 0.07	0.23

*Estimated from Figure 2a.

Mean pA_2 values and slopes of Schild plots are shown for both preparations in Table 3. The slopes of the Schild plots in some cases were significantly different from unity. This was most notable with prazosin. Increasing the contact time of prazosin with the tissue (from 30 to 60 min) had no significant effect on the slopes of the Schild plots. The order of potency of the antagonists in both tissues prazosin = BE 2254 > phentolamine > yohimbine > rauwolscine is indicative of an α_1 type of adrenoceptor. However the pA_2 values for prazosin (8.39), BE 2254 (7.90) and phentolamine (6.83) were all approximately one order of magnitude lower in portal veins than in aortic strips. Since the gradient of the Schild plots in some cases were significantly different from unity, pK_B values were calculated for each individual concentration of antagonist tested. None of these values was significantly different from the pA_2 values previously calculated.

pA_2 values were also calculated for prazosin and yohimbine using guanfacine and (−)-PE as agonists (Table 4). The pA_2 values of prazosin and yohimbine were not significantly different irrespective of which agonist was used.

Discussion

Postsynaptic α_2-adrenoceptors have been shown to exist in vascular smooth muscle (see Timmermans & Van Zweiten, 1981). Much of the evidence to support this view has come from experiments performed *in vivo*, rather than from *in vitro* experiments on isolated blood vessels. In the present study, a number of different agonist and antagonist drugs with varying specificity for α_1 and α_2-adrenoceptors have been used to characterize the receptors in rat isolated aortic strips and portal veins.

In rat aortic strips, (−)-NA, (−)-Adr (non selective), (−)-α-Me-NA and guanfacine (preferential α_2) and (−)-PE (preferential α_1) were all full agonists, while clonidine, oxymetazoline and 44,549 were partial agonists. Dissociation constants were calculated, after irreversible blockade of a proportion of the available receptors with Pbz (Furchgott, 1966; Furchgott & Bursztyn, 1967) and for the full agonists (−)-NA, (−)-PE and guanfacine were 11–29 times their respective EC_{50} values, whereas those for the partial agonists (calculated according to Waud, 1969 and Jenkinson, 1979) were similar to their pD_2 values. This would suggest in accord with previous studies (Ruffolo, Waddell & Yaden, 1980) that the rat aorta contains a large proportion of spare receptors, and that the partial agonists need to occupy a much greater proportion of the receptors than the full agonists in order to produce the same response.

In both pharmacological (Doxey, 1979) and radioligand studies (Summers, Jarrott & Louis, 1980), guanfacine has a high affinity and selectivity for α_2-adrenoceptors yet in rat aorta and portal vein it had low affinity, suggesting that in these preparations the receptors were of the α_1-type. (−)-NA (non-

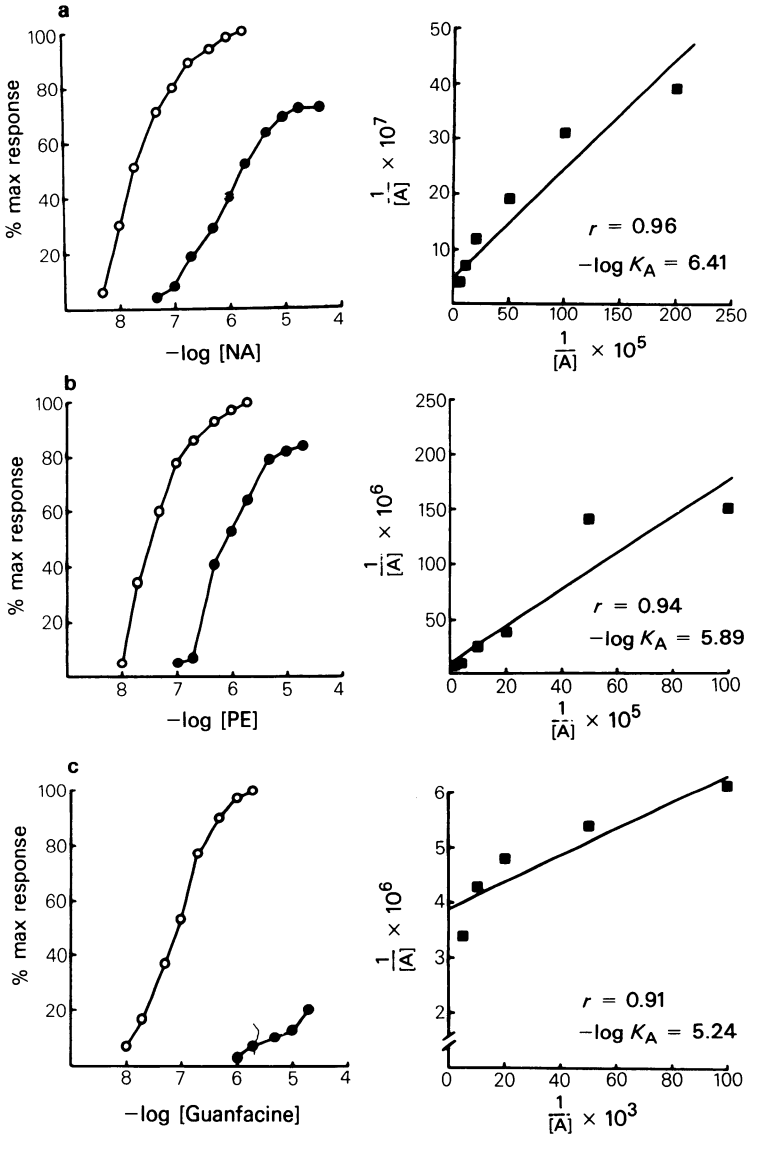

Figure 4 Estimation of K_A values for agonists in rat isolated aortic strips. In the left panels the effect of phenoxybenzamine (Pbz) (5×10^{-8} M) on the concentration-response curves to (−)-noradrenaline ((−)-NA) (a), (−)-phenylephrine ((−)-PE) (b), and guanfacine (c). In the right panels double reciprocal plots are shown of equiactive concentrations of the three agonists before [1/[A]] and after [1/[A']] phenoxybenzamine.

selective) and phenylephrine (α_1-selective) (Drew, 1977) were both more effective than (−)-α-Me NA (α_2-selective).

The order of potency of the antagonists (Table 4) is also indicative of an α_1-type of adrenoceptor. Both prazosin and yohimbine had similar pA_2 values irrespective of the agonist used (Table 3) suggesting that only a single population of adrenoceptors was present. The pA_2 for prazosin (approximately 9.5) is consistent with the values of 9.2 observed in guinea-pig splenic capsule (Digges, McPherson & Summers, 1981), 9.1–9.4 in rat anococcygeus (Doggrell & Paton, 1978) and 9.89 (pK_B) in rat mesenteric artery (Cohen, Wiley & Landry, 1980). Similarly, the pA_2 for yohimbine (approximately 6.7) in aortic strips is in close agreement with the values of 6.2 obtained in rabbit pulmonary artery (Borowski, Starke, Ehrl & Endo, 1977), 6.3 in rabbit spleen and aorta (Sheys &

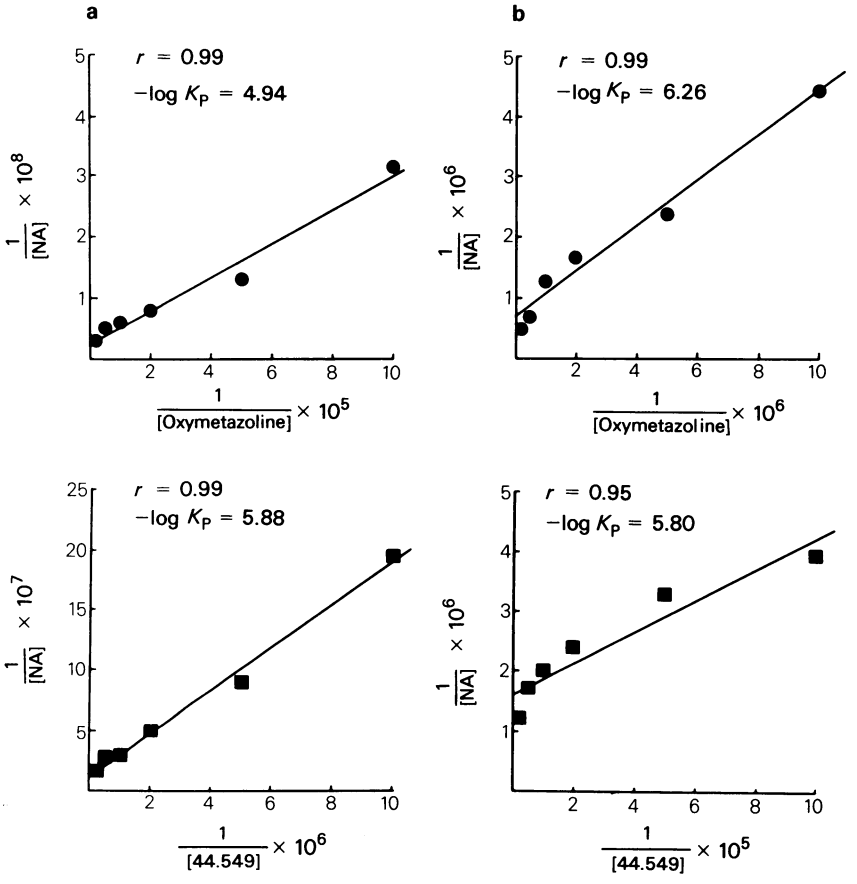

Figure 5 Estimation of K_A values for partial agonists in rat isolated aortic strips (a) and portal veins (b). Double reciprocal plots are shown of equiactive concentrations of (−)-noradrenaline ((−)-NA) against oxymetazoline (top panels) and 44,549 (bottom panels).

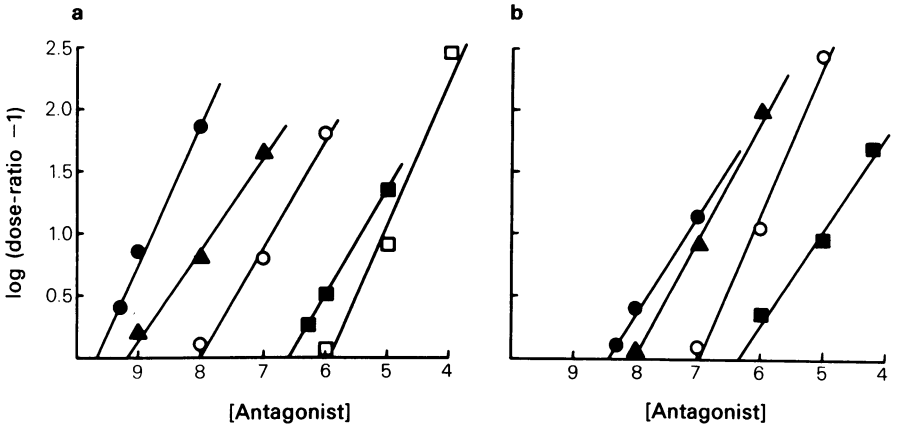

Figure 6 Arunlakshana & Schild plots (log (dose-ratio − 1) against − log antagonist concentration) for typical experiments in rat isolated aortic strips (a) or portal veins (b). Lines are shown for prazosin (●), BE 2254 (▲), phentolamine (○), yohimbine (■) and rauwolscine (□) antagonism of responses to (−)-noradrenaline.

Table 2 Dissociation constants of full agonists and partial agonists in rat isolated aortic strips and portal veins

Agonist	n	*Aortic strips*				*Portal veins*	
		K_A (M)	$-\log K_A$	K_p (M)	$-\log K_p$	K_p (M)	$-\log K_p$
Noradrenaline	7	$4.31 \pm 1.01 \times 10^{-7}$	6.41 ± 0.16	–	–	–	–
Phenylephrine	5	$7.46 \pm 3.05 \times 10^{-7}$	6.30 ± 0.18	–	–	–	–
Guanfacine	6	$1.02 \pm 0.22 \times 10^{-5}$	5.05 ± 0.10	–	–	$4.65 \pm 1.16 \times 10^{-5}$	4.40 ± 0.11
Clonidine	4	–	–	$1.30 \pm 0.66 \times 10^{-6}$	6.04 ± 0.21	$2.44 \pm 1.25 \times 10^{-6}$	4.85 ± 0.18
Oxymetazoline	5	–	–	$1.49 \pm 0.42 \times 10^{-5}$	4.88 ± 0.10	$7.57 \pm 2.55 \times 10^{-7}$	6.22 ± 0.19
44–549	5	–	–	$1.35 \pm 0.13 \times 10^{-6}$	5.88 ± 0.05	$2.52 \pm 1.25 \times 10^{-6}$	5.77 ± 0.18

Green, 1971), 6.4 in rat anococcygeus muscle (Doxey, Smith & Walker, 1977) and 7.1 in rat vas deferens (Eltze, 1979).

In the present experiments on aortic strips, in some cases, slopes of Schild plots were significantly greater than unity, most notably with prazosin using (−)-NA, (−)-PE or guanfacine as agonists. The reason for this remains unclear, since precautions were taken to prevent removal of agonists by uptake mechanisms. Schild plot slopes significantly different from 1 can also occur if the antagonist has not reached equilibrium (see Furchgott, 1972) but this does not appear to be the explanation here since no change occurred after increasing the contact time of prazosin with the tissue from 30 to 60 min. One possibility is that prazosin was not acting as a competitive antagonist as in rabbit blood vessels (Purdy, Krueger & Young, 1980).

In portal veins, (−)-NA, (−)-Adr, (−)-α-Me-NA and (−)-PE were full agonists, but were less potent than in aortic strips. Clonidine, oxymetazoline and 44,549 were partial agonists. Guanfacine which was a full agonist in aortic strips, was only a weak partial agonist in portal veins. This indicates fewer spare receptors in the portal veins than in the aortic strips.

In portal veins, the order of potency of the antagonist drugs (Table 4) was identical to that in aortic strips. pA_2 values of prazosin and pK_B values of rauwolscine were the same with either NA or PE as agonists (Table 3), again suggesting a single population of adrenoceptors. The order of potency of the antagonists would indicate that the receptors are of the α_1-type. However, although the mean pA_2 value for yohimbine was the same in both tissues, those of prazosin, BE 2254, phentolamine and rauwolscine (mean pK_B in portal veins) were all approximately one log unit less than in aortic strips. Thus, although the receptors in both tissues have the characteristics of α_1-adrenoceptors, the differences in the pA_2 values would suggest that the receptors may not be identical.

The present results in rat aortic strips indicate that the receptors resemble those found in hamster, dog and cat aorta and differ from those studied in rabbit and guinea-pig aorta and rat portal vein (Ruffolo, Waddell & Yaden, 1982). The lack of evidence for postsynaptic α_2-adrenoceptors in *in vitro* experiments on isolated blood vessels, poses a problem in explaining the location of the postsynaptic α_2-adrenoceptors shown to exist in *in vivo* experiments. Perhaps the most likely explanation is that the α_2-receptors are located on small resistance arterioles, which are more difficult to isolate and analyse in *in vitro* experiments.

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Table 3 pA_2 values, slopes of Schild plots of antagonists, using a selection of agonists, in the rat isolated aortic strips and portal veins

Tissue	*Antagonist*	*Agonist*	n	*pA_2*	*Slope of Schild plot*
Aortic strips	Prazosin	NA	7	9.42±0.07	1.42±0.11
		PE	6	9.60±0.05	1.13±0.13
		Guanfacine	6	9.62±0.11	1.33±0.08
	Yohimbine	NA	7	6.64±0.08	0.84±0.03
		PE	6	6.79±0.06	1.04±0.02
		Guanfacine	4	6.68±0.11	1.16±0.06
Portal veins	Prazosin	NA	6	8.39±0.13	0.84±0.06
		PE	6	8.17±0.18	0.93±0.07
	Rauwolscine	NA	12	5.26±0.12*	-
		PE	8	5.13±0.11*	-

*mean pK_B

Table 4 Mean pA_2 values, slopes of Schild plots and of antagonists, using (−)-noradrenaline as agonist, in rat isolated aortic strips and portal veins

		Aortic strips	
Antagonist	n	*pA_2*	*Slope of Schild plot*
Prazosin	7	9.42±0.07	1.42±0.11
BE 2254	6	9.42±0.07	0.70±0.02
Phentolamine	6	7.93±0.08	0.82±0.03
Yohimbine	7	6.64±0.08	0.84±0.03
Rauwolscine	8	6.44±0.15	1.03±0.09
		Portal veins	
Prazosin	6	8.39±0.13	0.84±0.08
BE 2254	6	7.90±0.09	1.07±0.06
Phentolamine	6	6.83±0.05	1.25±0.03
Yohimbine	8	6.42±0.08	0.72±0.02
Rauwolscine	12	5.26±0.12*	-

*mean pK_B

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–54.

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

BOROWSKI, E., STARKE, K., EHRL, H. & ENDO, T. (1977). A comparison of pre- and postsynaptic effects of α-adrenolytic drugs in the pulmonary artery of the rabbit. *Neuroscience*, **2**, 285–296.

COHEN, M.L., WILEY, K.S. & LANDRY, A.S. (1980). In vitro comparison of the pre- and postsynaptic alpha adrenergic receptor blocking properties of prazosin and tiodasozin (BL 5111). *Clin. exp. Hypert.*, **2**, 1067–1082.

DIGGES, K.G., McPHERSON, G.A. & SUMMERS, R.J. (1981). Pharmacological investigation of α-adrenoreceptors in guinea-pig splenic capsule. *J. auton. Pharmac.*, **1**, 313–320.

DIGGES, K.G. & SUMMERS, R.J. (1982). Comparison of α-adrenoceptors in aortic strips and portal veins of the rat. *Clin. exp. Pharmac. Physiol.* **9**, 403.

DOCHERTY, J.R., MacDONALD, A. & McGRATH, J.C. (1979). Further sub-classification of α-adrenoceptors in the cardiovascular system, vas deferens and anococcygeus muscle of the rat. *Br. J. Pharmac.*, **67**, 421–422P.

DOCHERTY, J.R. & McGRATH, J.C. (1980a). A comparison of pre- and post-junctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat: Evidence for two types of post-junctional alpha-adrenoceptor. *Naunyn Schmiedebergs Arch. Pharmac.*, **312**, 107–116.

DOCHERTY, J.R. & McGRATH, J.C. (1980b). An examination of factors influencing adrenergic transmission in the pithed rat, with special reference to noradrenaline uptake mechanisms and post-junctional α-adrenoceptors. *Naunyn Schmiedebergs Arch. Pharmac.*, **313**, 101–111.

DOCHERTY, J.R., CONSTANTINE, J.W. & STARKE, K. (1981). Smooth muscle of rabbit aorta contains α_1- but not α_2-adrenoceptors. *Naunyn Schmiedebergs Arch. Pharmac.*, **317**, 5–7.

DOCHERTY, J.R. & STARKE, K. (1981). Postsynaptic α-

adrenoceptor subtypes in rabbit blood vessels and rat anococcygeus muscle studied *in vitro*. *J. cardiovas. Pharmac.*, **3**, 854–866.

DOGGRELL, S.A. & PATON, D.M. (1978). Effects of prazosin and phentolamine on responses to field stimulation and to noradrenaline in vas deferens and anococcygeus muscle of the rat. *Archs int. Pharmacodyn.*, **233**, 209–220.

DOXEY, J.C. (1979). Pre- and postsynaptic effects of α agonists in the anoccoccygeus muscle of the pithed rat. *Eur. J. Pharmac.*, **54**, 185–189.

DOXEY, J.C., SMITH, C.F.C. & WALKER, J.M. (1977). Selectivity of blocking agents for pre- and postsynaptic α-adrenoceptors. *Br. J. Pharmac.*, **60**, 91–96.

DREW, G.M. (1977). Pharmacological characterisation of presynaptic α-adrenoceptors in the rat vas deferens. *Eur. J. Pharmac.*, **42**, 123–130.

DREW, G.M. & WHITING, S.B. (1979). Evidence for two distinct types of postsynaptic α-adrenoceptors in vascular smooth muscle *in vivo*. *Br. J. Pharmac.*, **67**, 207–215.

ELTZE, M. (1979). Investigations on the mode of action of a new anti-hypertensive drug, Urapidil, in the isolated rat vas deferens. *Eur. J. Pharmac.*, **9**, 1–9.

FURCHGOTT, R.F. (1966). The use of β-haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. *Adv. Drug Res.*, **3**, 21–55.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines, Handb. exp. Pharmac.*, vol. 33, ed. Blaschko, H. & Muscholl, E. pp. 283–335. Berlin: Springer-Verlag.

FURCHGOTT, R.F & BHADRAKOM, S. (1953). Reactions of strips of rabbit aorta to epinephrine, isoproterenol, sodium nitrite, and other drugs. *J. Pharmac. exp. Ther.*, **142**, 39–58.

FURCHGOTT, R.F. & BURSZTYN, P. (1967). Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors. *Ann. N.Y. Acad. Sci.*, **144**, 882–899.

GODFRAIND, T. & MILLER, R.C. (1982). α_1 and α_2 agonist actions on contraction and Ca fluxes in rat isolated aorta. *Br. J. Pharmac.*, **76**, 175P.

HAMILTON, C.A. & REID, J.L. (1980). Postsynaptic location of α_2-adrenoceptors in vascular smooth muscle. *Br. J. Pharmac.*, **70**, 63–64P.

JAUERNIG, R.A., MOULDS, R.F.W. & SHAW, J. (1978). The action of prazosin in human vascular preparations. *Archs int. Pharmacodyn.*, **231**, 81–89.

JENKINSON, D.H. (1979). Partial agonists in receptor classification. *Proceedings of the 6th International Symposium on Medicinal Chemistry*, pp. 373–383. Oxford: Cotswold Press.

LANGER, S.Z. (1974). Presynaptic regulation of catecholamine release. *Biochem. Pharmac.*, **23**, 1793–1800.

LANGER, S.Z., MASSINGHAM, R. & SHEPPERSON, N.B. (1981a). An explanation of the resistance of the noradrenaline pressor response to prazosin blockade *in vivo*. *Br. J. Pharmac.*, **72**, 121P.

LANGER, S.Z., MASSINGHAM, R. & SHEPPERSON, N.B. (1981b). Differential sensitivity to prazosin blockade of endogenously released and exogenously administered noradrenaline: possible relationship to the synaptic location of α_1- and the extrasynaptic location of α_2-adrenoceptors in dog vascular smooth muscle. *Br. J. Pharmac.*, **72**, 123P.

MADJAR, H., DOCHERTY, J.R. & STARKE, K. (1980). An examination of pre- and postsynaptic α-adrenoceptors in the autoperfused rabbit hindlimb. *J. cardiovasc. Pharmac.*, **2**, 619–627.

DE MEY, J.G. & VANHOUTTE, P.M. (1980). Differences in pharmacological properties of postjunctional alpha-adrenergic receptors among arteries and veins. *Archs int. Pharmacodyn.*, **244**, 328–329.

DE MEY, J.G. & VANHOUTTE, P.M. (1981). Uneven distribution of post-junctional alpha$_1$- and alpha$_2$-like adrenoceptors in canine arterial and venous smooth muscle. *Circulation Res.*, **48**, 875–884.

MOULDS, R.F.W. & JAUERNIG, R.A. (1977). Mechanisms of prazosin collapse. *Lancet*, **i**, 200–201.

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.

RUFFOLO, R.R., WADDELL, J.E. & YADEN, E.L. (1980). Receptor interactions of imidazolines. IV. Structural requirements for alpha adrenergic receptor occupation and receptor activation by clonidine and a series of structural analogs in rat aorta. *J. Pharmac. exp. Ther.*, **213**, 267–272.

RUFFOLO, R.R., WADDELL, J.E. & YADEN, E.L. (1982). Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. *Eur. J. Pharmac. exp. Ther.*, **221**, 309–314.

RUFFOLO, R.R., YADEN, E.L. & WADDELL, J.E. (1980). Receptor interactions of imidazolines. V. Clonidine differentiates postsynaptic alpha adrenergic receptor subtypes in tissues from the rat. *J. Pharmac. exp. Ther.*, **213**, 557–561.

SHEPPERSON, N.B. & LANGER, S.Z. (1981). The effects of the 2-amino tetrahydronapthalene derivative M7, a selective α_2 agonist in vitro. *Naunyn-Schmiedebergs Arch. Pharmac.*, **318**, 10–13.

SHEYS, E.M. & GREEN, R.D. (1972). A quantitative study of alpha-adrenergic receptors in the spleen and aorta of the rabbit. *J. Pharmac. exp. Ther.*, **180**, 317–325.

STARKE, K. (1981). α-Adrenoceptor subclassification. *Rev. Physiol. Biochem. Pharmac.*, **88**, 199–236.

SULLIVAN, A.T. & DREW, G.M. (1980). Pharmacological characterisation of pre- and postsynaptic α-adrenoceptors in dog saphenous vein. *Naunyn Schmiedebergs Arch. Pharmac.*, **314**, 249–258.

SUMMERS, R.J., JARROTT, B. & LOUIS, W.J. (1980). Selectivity of a series of clonidine-like drugs for α_1 and α_2 adrenoceptors in rat brain. *Neuroscience Letts.*, **20**, 347–350.

TIMMERMANS, P.B.M.W.M., KWA, H.Y. & VAN ZWEITEN, P.A. (1979). Possible subdivision of postsynaptic α-adrenoceptors mediating pressor responses in the pithed rat. *Naunyn Schmiedebergs Arch. Pharmac.*, **310**, 189–193.

TIMMERMANS, P.B.M.W.M. & VAN ZWEITEN, P.A. (1981). The postsynaptic α_2-adrenoceptor. *J. auton. Pharmac.*, **1**, 171–183.

WAUD, DR. (1969). On the measurement of the affinity of

partial agonists for receptors. *J. Pharmac. exp. Ther.*, **170,** 117–122.

WIKBERG, J.E.S. (1978). Pharmacological classification of adrenergic alpha-receptors in the guinea-pig. *Nature, Lond.*, **273,** 164–166.

WIKBERG, J.E.S. (1979). The pharmacological classification of adrenergic α_1- and α_2-receptors and their mechanisms of action. *Acta physiol. scand.*, Suppl. **468.**

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