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Pharmacological characterization of noradrenaline-induced contractions of the porcine isolated palmar lateral vein and palmar common digital artery

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1 The aim of this study was to examine the pharmacological characteristics of α-adrenoceptor-mediated contractions in two porcine isolated blood vessels, the palmar lateral vein (PLV) and the palmar common digital artery (PCDA). This was carried out with noradrenaline used as the agonist throughout, and either phentolamine (non-selective α-adrenoceptor antagonist), prazosin and YM-12617 (selective α_1-adrenoceptor antagonists) or rauwolscine and CH-38083 (selective α_2-adrenoceptor antagonists).

2 Noradrenaline (0.003–10 μM) produced concentration-dependent contractions in both vessels, with the PCDA ($pD_2 = 6.33 \pm 0.07$, $n = 10$) being approximately 10 fold less sensitive to noradrenaline compared to the PLV ($pD_2 = 7.39 \pm 0.09$, $n = 8$). Also, the maximal response to noradrenaline was greater in the PCDA compared to the PLV. Phentolamine (0.03–30 μM) produced parallel rightward shifts in the CRC to noradrenaline in both tissue preparations. The pA_2 values were similar and slopes of the Schild plots were not significantly different from unity, indicating an interaction between phentolamine and a single receptor in each preparation.

3 In the PCDA the α_1-adrenoceptor antagonists, prazosin (0.01–1 μM) and YM-12617 (0.01–1 μM) produced non-parallel rightwards shifts in the CRC to noradrenaline, with the lower 10–15% of the CRC exhibiting greater resistance to the effects of these antagonists compared to the upper part. In contrast, rauwolscine (1–10 μM) and CH-38083 (10 μM) produced parallel displacement of the CRC to noradrenaline. In the PLV, low concentrations of either α_1- (0.01 μM) or α_2-adrenoceptor antagonists (0.1–1 μM) produced a large shift in the CRC, but subsequent higher concentrations had only small additional effects. Based upon pK_B values estimated from the effects of the lower concentrations of antagonists, the results are consistent with a large population of α_1-adrenoceptors in the PCDA and a mixture of α_1- and α_2-adrenoceptors in the PLV.

4 In both tissues, when an α_1- and an α_2-adrenoceptor antagonist were used in combination the effect produced was greater than that with either agent alone. In contrast, the combination of the α_1-adrenoceptor antagonists (prazosin and YM-12617 together) or the α_2-adrenoceptor antagonists (CH-38083 and rauwolscine together) were no more effective than that produced by the individual antagonists. These findings suggest the presence of functional α_1- and α_2-adrenoceptors in the PLV and PCDA.

5 Phenoxybenzamine (0.3–3 μM, 60 min exposure) produced a concentration-dependent reduction in the maximal response to noradrenaline which was more pronounced in the PCDA than the PLV. After a 60 min exposure to a combination of phenoxybenzamine (1 μM) and rauwolscine (1 μM), the remaining NA-induced contraction after washout was resistant to prazosin (0.1 μM) and sensitive to rauwolscine (1 μM) in both tissue preparations, indicating the existence of functional α_2-adrenoceptors in both vessels.

6 Evidence suggests that post-junctional α_1- and α_2-adrenoceptors contribute to noradrenaline-induced contractions in the PCDA and PLV, with the latter possessing a larger population of functional α_2-adrenoceptors.

Keywords: Noradrenaline; porcine vascular smooth muscle; α_1-adrenoceptors; α_2-adrenoceptors; contraction

Introduction

Over the past 40 years, experimental studies on isolated blood vessels from standard laboratory animals, e.g. rat, rabbit, guinea-pig and, less commonly, cat, dog and primates, have yielded invaluable information on the properties of vascular smooth muscle. Although this information has been useful in the development of therapeutic agents for man, it is increasingly evident that the pharmacological characteristics vary between and also within individual vascular beds. The problem is further compounded by findings that even for the same vessel from different species, e.g. the thoracic aorta or mesenteric artery, substantial differences exist in the degree of sympathetic innervation (Patil *et al.*, 1972), utilization of cellular calcium and extracellular calcium for contraction (Beckeringh *et al.*, 1984; Jenkin *et al.*, 1991) and the pharmacological characteristics of α-adrenoceptors (Ruffolo & Waddell, 1982; Ruffolo *et al.*, 1982; Nielsen *et al.*, 1991). In view of the difficulty associated with access and viability of non-diseased human blood vessels, it is clearly important to widen the range of isolated blood vessels available for experimental studies and ensure that these adequately reflect the heterogeneous characteristics of vascular smooth muscle. There are several reasons to believe that the pig may be a useful model for this purpose.

First, porcine aortic and mitral valves have been successfully employed in man for over 15 years (Burdon *et al.*, 1992) and it has been suggested that pigs, genetically engineered to reduce hyperacute rejection, may eventually be used for xenotransplantation (First, 1992; Good *et al.*, 1992; Cooper 1992). Secondly, pulmonary (Kovitz *et al.*, 1993), renal

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(Christie & Lewis, 1991), carotid (Ohgushi *et al.*, 1993), cerebral (Linnik & Lee, 1989; Kim *et al.*, 1992) and, in particular, coronary blood vessels (Shafiq *et al.*, 1992; Ohgushi *et al.*, 1993; Ito *et al.*, 1993) from the pig have been successfully used to examine the properties of vascular smooth muscle. Finally, the availability of large quantities of tissue has enabled radioligand binding and biochemical studies to be conducted on both aortic and coronary vascular smooth muscle (Nishimura *et al.*, 1987; Tsutsui *et al.*, 1990; Ito *et al.*, 1993), and it seems possible that this could be extended to other blood vessels.

In the present study we have focused on an examination of the pharmacological characteristics of α-adrenoceptor-mediated contraction of the porcine isolated palmar lateral vein (PLV) and palmar common digital artery (PCDA) to assess the suitability of the pig as a (cardiovascular) model for man. The corresponding human vessels were amongst the first shown to possess a population of constrictor α_2-adrenoceptors (Stevens & Moulds, 1981), while demonstration of this subtype in superficial vessels from standard laboratory animals often requires an ancillary spasmogen (rat tail; Templeton *et al.*, 1989), extensive pharmacological manipulation (rabbit isolated saphenous vein; Daly *et al.*, 1988c) or a combination of both (rabbit saphenous artery; Dunn *et al.*, 1991). We have used noradrenaline as the agonist throughout and examined the effect of prazosin and YM-12617, antagonists selective for α_1-adrenoceptors (Honda *et al.*, 1985; McGrath *et al.*, 1989), and rauwolscine and CH-38083, antagonists selective for α_2-adrenoceptors (Weitzell *et al.*, 1979; Vizi *et al.*, 1986). Our results indicate that the PLV and PCDA possess a population of α_1- and α_2-adrenoceptors which are stimulated by noradrenaline to produce a contraction.

Methods

Preparation of the blood vessel

Porcine trotters from the forelimbs were obtained within 30 min of death of the animal, placed on ice and transported to the laboratory. A 3–4 cm length of the palmar common digital artery and palmar lateral vein (Ghosal & Nanda, 1975) was dissected out and stored overnight at 4°C in modified Krebs-Henseleit saline containing 2% Ficoll which had been previously gassed with 95% O_2/5% CO_2. The composition of the modified Krebs-Henseleit saline was (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2 and glucose 11.1. Previous experiments have established that this procedure neither impairs the contractility of the smooth muscle nor the ability of the endothelium to release nitric oxide (Wellman *et al.*, 1993; Lot & Wilson, 1994).

Isometric tension recordings

On the following day, the vein and artery were carefully cleaned of connective tissue and divided into 5 mm ring segments. Stainless steel wire (0.2 mm thick) supports were inserted into the lumen and each segment suspended in an isolated organ bath containing modified Krebs-Henseleit saline with 10 μM cocaine (to inhibit uptake$_1$), 1 μM propranolol (to inhibit β-adrenoceptors) and Na_2EDTA (23 μM) to prevent the oxidative degradation of noradrenaline. The Krebs-Henseleit saline was maintained at 31°C, as this has been shown to enhance the function of postjunctional α_2-adrenoceptors (Flavahan & Vanhoutte, 1986; Templeton *et al.*, 1989), and gassed with 95% O_2 and 5% CO_2. No attempt was made to remove the endothelium. The lower support was fixed, and the upper support was connected to a Grass FT-03 transducer linked to a Grass Polygraph. After 30 min equilibration in the Krebs-Henseleit saline, approximately 8 g wt. tension (artery) and 4 g wt. tension (vein) were slowly applied and the tissue allowed to relax to a final resting tension of 1.5–2 g wt. (artery) and 0.5–0.7 g wt. (vein). After 60 min equilibration, each preparation was exposed to 1 μM (−)-noradrenaline (NA) and allowed to contract for 5 min. Following complete washout, the preparation was again challenged with 1 μM noradrenaline, washed and an additional 1 h equilibration period allowed before starting the experiment. This procedure was found to minimize changes in the sensitivity of the preparation to further addition of noradrenaline. In experiments involving phenoxybenzamine the preparations were repeatedly exposed to 40 mM KCl, rather than noradrenaline, to establish reproducible responses.

In all experiments cumulative concentration-response curves (CRC) were constructed by exposing the tissue to increasing concentrations (0.5 log unit increments) of the agonist until a maximum response was observed. Since contractile responses of the palmar lateral vein to the α-adrenoceptor agonists were not well maintained over a period greater than 90 s, the addition of the next concentration of the agonist was made as close to the peak response as possible. For the palmar common digital artery, the CRC was terminated at 3 μM noradrenaline to avoid receptor desensitization. Successive CRCs were separated by 60 min, as measured from the time following washout and complete relaxation of the preparation, and a maximum of three CRCs was constructed in each preparation.

The effect of reversible α-adrenoceptor antagonists

The α-adrenoceptor antagonists, phentolamine (0.03–30 μM), prazosin (0.01–1 μM), rauwolscine (0.1–10 μM), CH-38083 (0.1–10 μM), YM-12617 (0.01–1 μM) or a combination of the subtype-selective antagonists were added at least 40 min prior to the construction of a second or third CRC. In one series of experiments, the effect of phentolamine against noradrenaline-induced contractions was also examined in the presence of 10 μM corticosterone (to inhibit extraneuronal uptake).

The effect of phenoxybenzamine

Preparations were exposed to a single concentration of phenoxybenzamine (0.3 μM, 1 μM or 3 μM) or saline for 60 min (after reproducible responses to KCl had been established) and then washed a minimum of five times over the next 60 min before exposure to increasing concentrations of noradrenaline. In another series of experiments, preparations were exposed to 1 μM rauwolscine 5 min before the addition of 1 μM phenoxybenzamine to increase the degree of protection for α_2-adrenoceptors (Daly *et al.*, 1988c). After 60 min, the phenoxybenzamine was removed by washing twice with Krebs-Henseleit saline containing 1 μM rauwolscine and this antagonist was removed 5 min later (total time; 60 min for phenoxybenzamine, 70 min for rauwolscine). The preparations were washed a further five times over 45 min and a concentration-response curve to noradrenaline constructed. The noradrenaline CRC was repeated 60 min later after 40 min exposure to either 0.1 μM prazosin, 1 μM rauwolscine or vehicle.

Data analysis

The sensitivity of the preparations to noradrenaline was assessed as the negative logarithm of the concentration required to cause 50% of the maximum response (pD_2). The agonist concentration-ratio in the presence and absence of the antagonist was determined at level of either 50% of the maximum response (palmar lateral vein) or 50% of the control response to 3 μM noradrenaline (palmar common digital artery). Using the agonist concentration-ratio produced by the lowest effective concentration of the antagonist, an estimate of the negative logarithm of the dissociation con-

stant (pK_B) was determined by the method of Furchgott (1972). In the case of phentolamine, a pA_2 value was determined by the method of Arunlakshana & Schild (1959).

In all experiments, one preparation was run in parallel with the experimental tissues, but received no antagonist, and was used to determine time-dependent changes in agonist sensitivity (Furchgott, 1972). All responses are expressed as a percentage of the maximum response and given as the mean ± s.e. mean. Differences between means were considered statistically significant if $P<0.05$ for unpaired or paired observations (Student's t test). The logarithm of the concentration of noradrenaline producing either 25% or 50% of the control maximum response or agonist-concentration ratios was calculated with the logistic equation described by DeLean *et al.* (1978) with Kalidegraph software (Synergy) on a MacIntosh LC II computer.

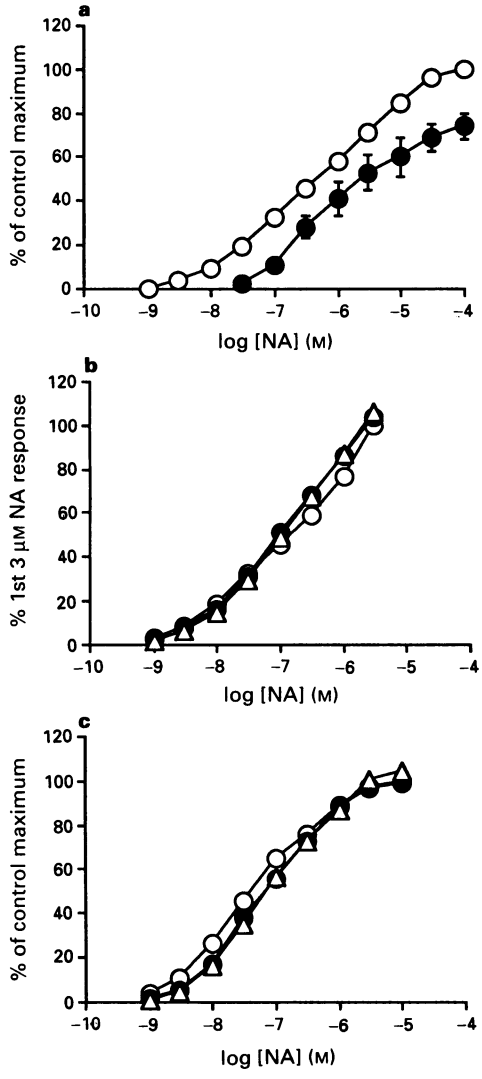

Figure 1 The reproducibility of noradrenaline (NA) concentration-response curves (CRCs) in the porcine isolated palmar common digital artery (a and b) and palmar lateral vein (c). The highest concentration of noradrenaline employed was 100 μM (a), 3 μM (b) and 10 μM (c), and (○) denotes the 1st CRC, (●) denotes the 2nd CRC and (△) denotes the 3rd CRC. All points represent the mean of 6 observations (a) or 9 observations (b and c) with the s.e. mean in (a). In (b) and (c) the s.e. mean, which were less than 6%, have been omitted to improve clarity.

Drugs

The following compounds were used: (−)-noradrenaline bitartrate (Sigma); prazosin HCl (Pfizer); rauwolscine HCl (Roth); YM-12617 (5-[2-[[2-(ethoxyphenoxy)ethyl]amino] propyl]-2-methoxy benzene-sulphonamide HCl) (Yamanouchi); CH-38083 (7,8-(methylenedioxi)-14-α-hydroalloberbane HCl) (Chinoin); phenoxybenzamine HCl (SKB); corticosterone (Sigma); phentolamine mesylate (Rogitine, Ciba Geigy); propranolol HCl (Sigma); cocaine HCl (MacCarthys), Ficoll 70,000 (Sigma). Stock solutions of noradrenaline were prepared in distilled water with 23 μM Na_2EDTA. Prazosin (1 mM) was dissolved in 0.1 M lactic acid and dilutions made in distilled water. Phenoxybenzamine (1 mM) was prepared in 20% absolute alcohol in distilled water and a drop of 1 N HCl to remove turbidity and further dilutions were made in distilled water. Corticosterone (10 mM) was dissolved in propylene glycol. All other drugs were dissolved in distilled water and added to the organ baths in a volume of 0.1 ml or less.

Results

The reproducibility of responses to noradrenaline and the effect of phentolamine

In the presence of 10 μM cocaine and 1 μM propranolol, noradrenaline (0.001–100 μM) produced concentration-dependent contractions of the PCDA and the PLV (Figure 1a,b). The maximum response of the PCDA was greater than that of the PLV, but the sensitivity of the arterial preparation to noradrenaline was 1/10th of that of the vein (Table 1). The addition of 10 μM corticosterone to the bathing medium caused a 2 fold increase in the sensitivity of the PCDA to noradrenaline, but did not alter the sensitivity of the PLV (Table 1).

Exposure of the PCDA to 100 μM noradrenaline (in order to achieve the maximum effect) required 2 h of repeated washing to effect complete relaxation of the smooth muscle. As shown in Figure 1a, the subsequent CRC was displaced approximately 3 fold to the right (pD_2 – 5.95 ± 0.13, $n=6$) and was associated with a 20–25% reduction in the max-

Table 1 Responses of the porcine isolated palmar common digital artery (PCDA) and palmar lateral vein (PLV) to noradrenaline

	Force (g wt)	pD_2
PCDA		
Cocaine 10 μM, propranolol 1 μM	10.4 ± 0.9 ($n=10$)	6.33 ± 0.07 ($n=10$)
Cocaine 10 μM, propranolol 1 μM, corticosterone 10 μM	9.2 ± 0.8 ($n=8$)	6.63 ± 0.08 ($n=8$)*
PLV		
Cocaine 10 μM, propranolol 1 μM	3.24 ± 0.22 ($n=8$)	7.39 ± 0.09 ($n=8$)
Cocaine 10 μM, propranolol 1 μM, cortisterone 10 μM	2.88 ± 0.19 ($n=8$)	7.48 ± 0.12 ($n=8$)

Values shown are the mean ± s.e.mean of n experiments (shown in parentheses). *Significantly different from value in the absence of corticosterone ($P<0.05$, unpaired t test).

imum response. Inclusion of 10 μM corticosterone to the bathing medium, reduced the time required to effect complete relaxation of the PCDA following exposure to 100 μM noradrenaline, but did not appreciably alter the apparent desensitization. By limiting the highest concentration of noradrenaline to 3 μM for the PCDA and 10 μM for the PLV, three reproducible CRCs could be constructed 75 min apart (Figure 1b,c).

Figure 2 shows the effect of phentolamine against noradrenaline-induced contractions of the PCDA and PLV in the presence of 10 μM cocaine and 1 μM propranolol. Phentolamine (0.03–30 μM) produced a parallel rightward displacement of the concentration-response curves for noradrenaline in both preparations. As shown in Table 2, the pA_2 value for phentolamine was similar in both preparations and the slopes of the Schild plots were not significantly different from unity. Neither the pA_2 value nor the slope of the Schild plot for phentolamine was significantly altered by the inclusion of 10 μM corticosterone in the bathing medium (Table 2). Since, corticosterone failed to alter the interaction between phentolamine and noradrenaline at α-adrenoceptors in either preparation, it was decided to conduct all subsequent experiments in the presence of only 10 μM cocaine and 1 μM propranolol.

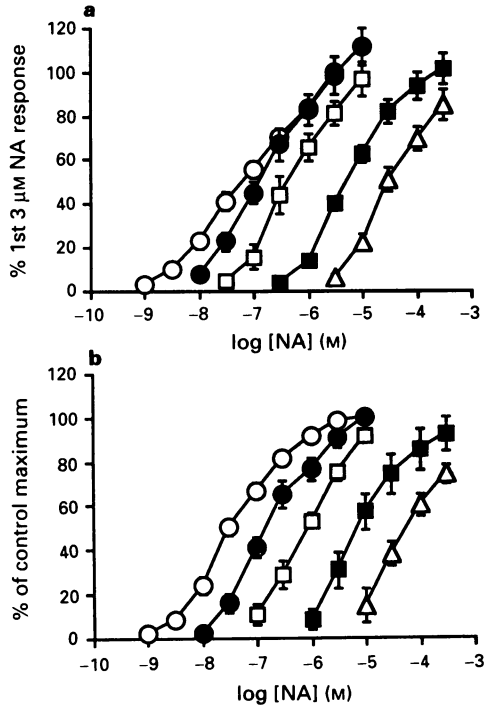

Figure 2 The effect of 0.03 μM (●), 0.3 μM (□), 3 μM (■) and 30 μM (△) phentolamine against noradrenaline (NA)-induced contraction of (a) the porcine isolated palmar common digital artery and (b) the porcine isolated palmar lateral vein. The control concentration-response curve to noradrenaline is represented by (○). All points represent the mean ± s.e. mean of 6 observations.

The effect of other α-adrenoceptor antagonists against noradrenaline-induced contractions

Figure 3 shows the effect of prazosin, YM-12617, rauwolscine and CH-38083 against noradrenaline-induced contractions in the PCDA. Prazosin (0.01–1 μM) and YM-12617 (0.01–1 μM) produced a non-parallel rightward displacement of noradrenaline CRCs, with the lower 10–15% of the CRC exhibiting greater resistance to the effect of these antagonists than the upper part of the CRC (Figure 3a, b). In contrast, rauwolscine (1–10 μM) and CH-38083 (10 μM) produced a parallel displacement of the noradrenaline CRC; lower concentrations of the antagonists failed to produce any effect. In the PLV, the lowest concentration of prazosin (0.01 μM) and YM-12617 (0.01 μM) examined produced a 10–20 fold rightward displacement of the noradrenaline CRC (Figure 4a,b). However, further increases in the concentration of the antagonists (to 1 μM) produced only a small additional displacement of the CRC. Qualitatively similar profiles were obtained for rauwolscine (0.1–10 μM) and CH-38083 (0.1–10 μM) against noradrenaline-induced contractions of the PLV (Figure 4c,d).

Based upon the displacement of the CRC produced by the lowest effective concentration of the antagonists (Table 3), the estimated -log K_B values for the antagonists are consistent with a large population of α_1-adrenoceptors in the PCDA and a mixture of α_1- and α_2-adrenoceptors in the PLV.

The effect of combinations of the α-adrenoceptor antagonists

Concentrations of the antagonists which produced a 15–50 fold rightward displacement of the noradrenaline CRC in PLV (see Table 4) were chosen in order to examine the effects of combinations of the antagonists against noradrenaline-evoked contractions in both preparations. Figure 5 shows that the combination of 1 μM prazosin and 1 μM rauwolscine produced a significantly greater rightward displacement of the noradrenaline CRC in the PCDA and PLV than either 1 μM rauwolscine and 10 μM CH-38083 in combination, or 1 μM prazosin and 0.1 μM YM-1267 in combination. In both cases, prazosin and rauwolscine caused more than a 1000 fold rightward displacement of the

Table 2 pA_2 values and slope for the Schild plot (with 95% confidence intervals) for phentolamine, in the presence of various ancillary agents, against noradrenaline-induced contractions of the porcine isolated palmar lateral vein (PLV) and palmar common digital artery (PCDA)

		(n)	pA_2	Slope
PLV	Cocaine 10 μM, propranolol 1 μM	19	7.78 (8.26–7.26)	0.91 (1.03–0.70)
	Cocaine 10 μM, propranolol 1 μM, corticosterone 10 μM	20	8.06 (8.41–7.70)	0.92 (1.05–0.78)
PCDA	Cocaine 10 μM, propranolol 1 μM	19	7.56 (8.08–7.04)	0.80 (1.14–0.68)
	Cocaine 10 μM, propranolol 1 μM, corticosterone 10 μM	17	7.52 (7.19–7.14)	0.88 (1.15–0.77)

The values shown are based on a minimum of 4 observations at each of 4 observations at each of 4 concentrations (0.03 μM, 0.3 μM, 3 μM and 30 μM) of phentolamine.

noradrenaline CRC; an effect greater than either agent alone (Table 4). In contrast, the combination of 1 μM prazosin with 0.1 μM YM-12617 was no more effective than either agent alone. Table 4 also shows that in both preparations the combination of 0.1 μM YM-12617 and 10 μM CH-38083 was more effective than either agent alone.

Attempts to isolate functional α_2-adrenoceptors

The above findings indicate that a combination of a selective α_1-adrenoceptor antagonist (prazosin or YM-12617) with a selective α_2-adrenoceptor antagonist (rauwolscine or CH-38083) is more effective than the combination of antagonists

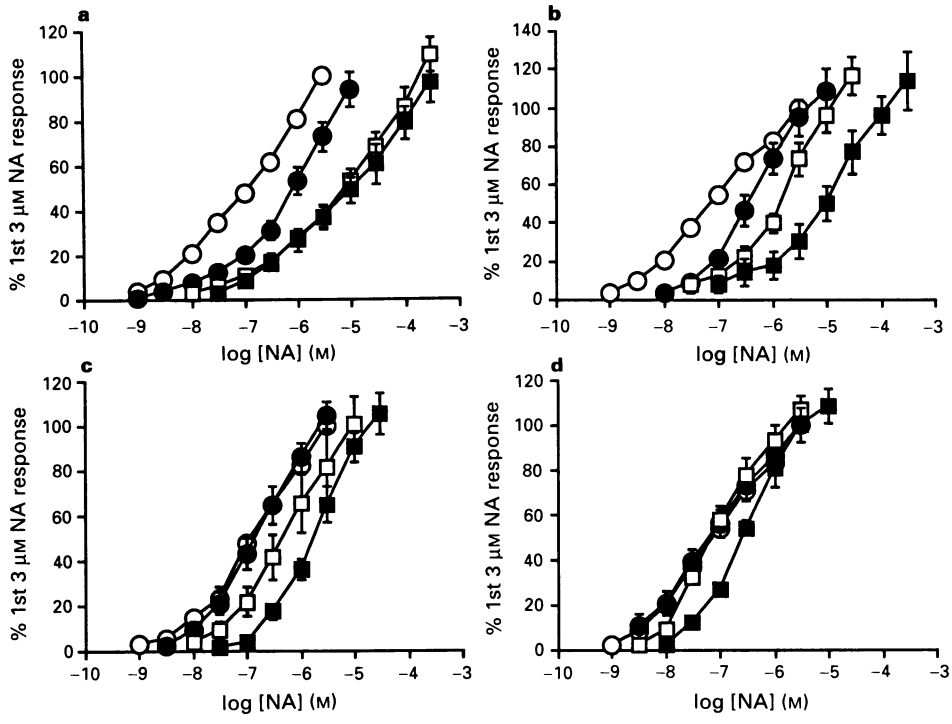

Figure 3 The effect of (a) 0.01 μM (●), 0.1 μM (□), 1 μM (■) prazosin; (b) 0.01 μM (●), 0.1 μM (□), 1 μM (■) YM-12617; (c) 0.1 μM (●), 1 μM (□), 10 μM (■) rauwolscine and (d) 0.1 μM (●), 1 μM (□) 10 μM (■) CH-38083 against noradrenaline (NA)-induced contractions of the porcine isolated palmar common digital artery. The control concentration-response curve to noradrenaline is represented by (○). All points represent the mean ± s.e. mean of 5–7 observations.

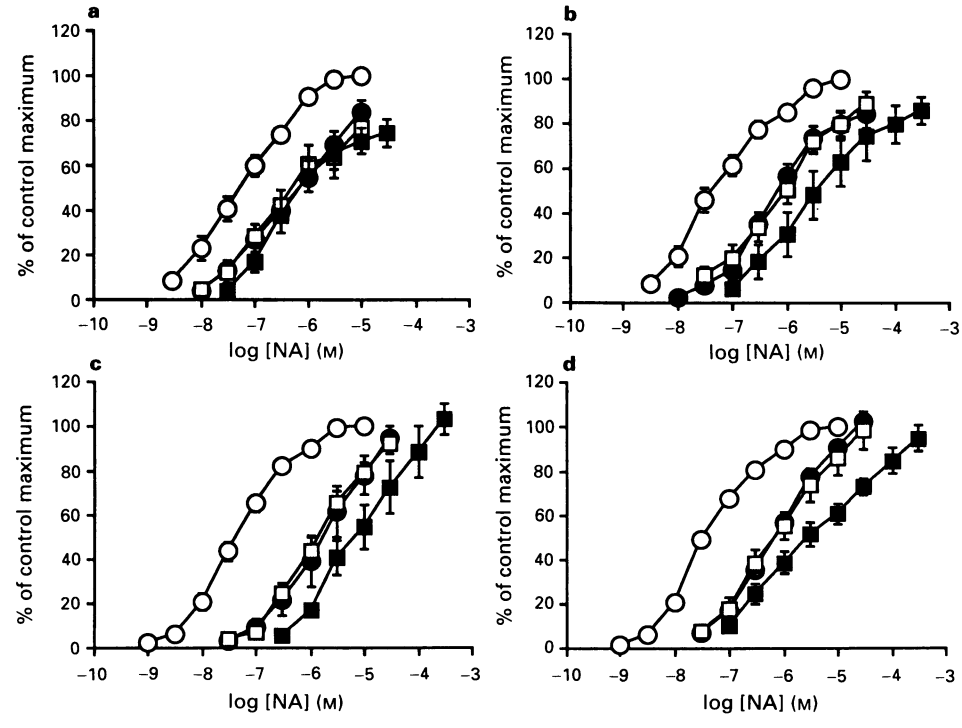

Figure 4 The effect of (a) 0.01 μM (●), 0.1 μM (□), 1 μM (■) prazosin; (b) 0.01 μM (●), 0.1 μM (□), 1 μM (■) YM-12617; (c) 0.1 μM (●), 1 μM (□), 10 μM (■) rauwolscine and (d) 0.1 μM (●), 1 μM (□) 10 μM (■) CH-38083 against noradrenaline (NA)-induced contractions of the porcine isolated palmar lateral vein. The control concentration-response curve to noradrenaline is represented by (○). All points represent the mean ± s.e. mean of 5–10 observations.

from the same group. This suggests that α_1- and α_2-adrenoceptors contribute to responses to noradrenaline in both preparations. Confirmation of this provided by the results in Figure 6. Figure 6a and b shows the effects of a 60 min exposure to phenoxybenzamine on contractions to noradrenaline in the PCDA and PLV, respectively. Phenoxybenzamine (0.3–1 μM) produced a concentration-dependent reduction in the maximum response to noradrenaline, which was more pronounced in the PCDA (Figure 6a) than in the PLV (Figure 6b). Phenoxybenzamine 3 μM practically abolished noradrenaline-induced contractions.

The α-adrenoceptor(s) contributing to the response to the noradrenaline remaining after exposure to a combination of 1 μM phenoxybenzamine and 1 μM rauwolscine was examined by investigating the effect of 0.1 μM prazosin and 1 μM rauwolscine. Figure 6c shows that 0.1 μM prazosin failed to affect responses to low concentrations of noradrenaline (0.03–1 μM) in the PCDA, but reduced responses to high concentrations of noradrenaline (> 1 μM). Rauwolscine 1 μM produced only a 10 fold rightward displacement of noradrenaline CRC in the PCDA. In contrast, 0.1 μM prazosin failed to affect responses to noradrenaline in the PLV, while 1 μM rauwolscine produced a 300 fold rightward displacement of the noradrenaline CRC (Figure 6d).

Table 3 Mean $-\log K_B$ values (± s.e.mean) for several antagonists at α-adrenoceptors in the porcine isolated palmar common digital artery (PCDA) and palmar lateral vein (PLV)

	PCDA	*PLV*
Prazosin	8.83 ± 0.09 (0.01 μM, $n = 9$)	8.73 ± 0.25 (0.01 μM, $n = 6$)
YM-12617	8.96 ± 0.22 (0.01 μM, $n = 6$)	8.63 ± 0.14 (0.01 μM, $n = 6$)
Rauwolscine	6.60 ± 0.22 (1.1 μM, $n = 5$)	8.31 ± 0.22 (0.1 μM, $n = 10$)
CH-38083	5.22 ± 0.08 (10 μM, $n = 5$)	8.20 ± 0.13 (0.1 μM, $n = 5$)
Comment: major α-adrenoceptor subtype	α_1	α_1 and α_2

Values shown are based on the shift in the concentration-response curve produced by the lowest effective concentration of the antagonist, measured at the 50% level of either the maximum control response (PLV) or the control response to 3 μM noradrenaline (PCDA). The concentration of the antagonist used for calculating the $-\log K_B$ value and the number of experimental observations are shown in parentheses.

Discussion

As indicated in the Introduction, the principal objective of this study was to examine the pharmacological characteristics of α-adrenoceptors mediating contractions of the PCDA and PLV and assess the suitability of these blood vessels as a model for man. The approach adopted during the course of this investigation was to use noradrenaline as the agonist, because it does not discriminate between α_1- and α_2-

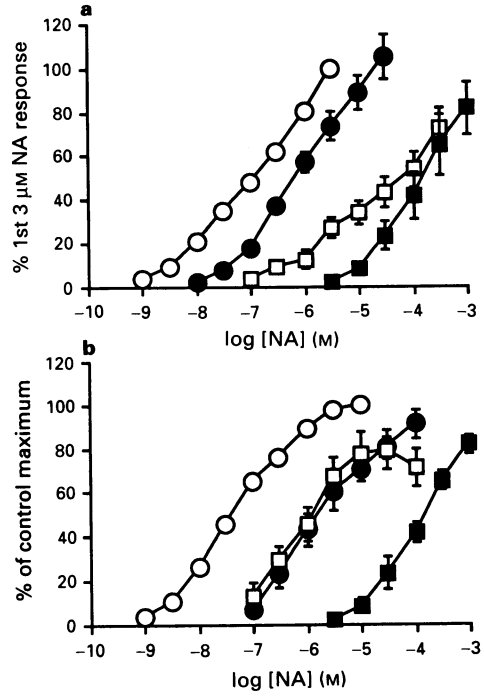

Figure 5 The effect of 1 μM rauwolscine and 10 μM CH-38083 (●); 0.1 μM YM-12617 and 1 μM prazosin (□); 1 μM prazosin and 1 μM rauwolscine (■) against noradrenaline (NA)-induced contractions in (a) porcine isolated palmar common digital artery and (b) porcine isolated palmar lateral vein. The control concentration-response curve to noradrenaline is represented by (○). All points represent the mean ± s.e. mean of 6–9 observations.

Table 4 Logarithm of the concentration-ratio for noradrenaline produced by 'selective' concentrations of the antagonists, either alone or in combination, in the porcine isolated palmar lateral vein (PLV) and palmar common digital artery (PCDA)

	PCDA	*PLV*
CH-38083* 10 μM	0.71 ± 0.10 ($n = 5$)	1.75 ± 0.13 ($n = 10$)
Rauwolscine* 1 μM	0.66 ± 0.22 ($n = 5$)	1.17 ± 0.08 ($n = 9$)
Combination	0.80 ± 0.15 ($n = 7$)	1.58 ± 0.11 ($n = 7$)
Prasozin* 1 μM	1.52 ± 0.18 ($n = 6$)	1.16 ± 0.19 ($n = 9$)
YM-12617* 0.1 μM	1.61 ± 0.14 ($n = 6$)	1.35 ± 0.18 ($n = 9$)
Combination	1.98 ± 0.17 ($n = 7$)	1.45 ± 0.14 ($n = 7$)
YM-12617* 0.1 μM	1.61 ± 0.14 ($n = 6$)	1.35 ± 0.18 ($n = 9$)
CH-38083* 10 μM	0.71 ± 0.22 ($n = 5$)	1.75 ± 0.13 ($n = 10$)
Combination	2.47 ± 0.21 ($n = 7$)†	2.50 ± 0.18 ($n = 7$)†
Prazosin* 1 μM	1.52 ± 0.18 ($n = 6$)	1.16 ± 0.19 ($n = 9$)
Rauwolscine* 1 μM	0.66 ± 0.22 ($n = 5$)	1.17 ± 0.08 ($n = 9$)
Combination	3.21 ± 0.23 ($n = 6$)†	3.38 ± 0.14 ($n = 9$)†

Values shown are the mean ± s.e.mean of n experiments (shown in parentheses). *Concentration-ratio calculated at the 50% level of the maximum control response to noradrenaline (PLV) or at the level of 25% of the control response to 3 μM noradrenaline (PCDA). *Values taken from a separate series of experiments (see Figures 3 and 4). †The effect of the combination of the antagonists is significantly more effective than either antagonist alone ($P < 0.05$. Student's unpaired t test).

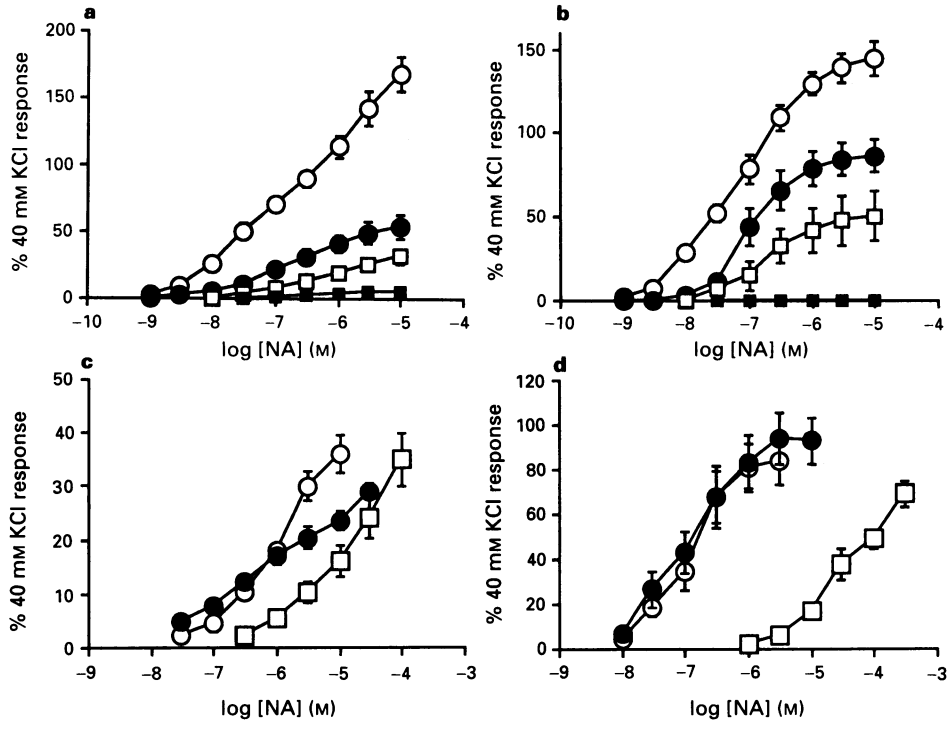

Figure 6 (a, b) The effect of prior exposure to 0.1 μM (●), 1 μM (□) and 3 μM (■) phenoxybenzamine (60 min exposure followed by 45 min washout) on noradrenaline (NA)-induced contractions of the (a) the porcine isolated palmar common digital artery and (b) porcine isolated palmar lateral vein. The control concentration-response curve to noradrenaline is represented by (○). All points represent the mean of 5–6 observations ± s.e. mean. (c,d) The effect of 0.1 μM prazosin (●) and 1 μM rauwolscine (□) against noradrenaline (NA)-induced contractions of the (c) the porcine isolated palmar common digital artery and (d) the porcine isolated palmar lateral vein, after exposure to a combination of 1 μM phenoxybenzamine (60 min) and 1 μM rauwolscine (70 min) followed by a several washes over 45 min. The control concentration-response curve to noradrenaline is represented by (□). All points represent the mean ± s.e. mean of 5–6 observations.

adrenoceptors (McGrath 1982; McGrath *et al.*, 1989), and rely upon selective antagonists to establish the presence of particular receptor subtypes. Support for this stance is provided by Sjöberg and coworkers (1987), who observed that B-HT 920 and clonidine, agonists which selectively activate α_2-adrenoceptors in other species, failed to elicit contractions of human blood vessels while the combination of noradrenaline and a selective α_1-adrenoceptor antagonist suggested the presence of postjunctional α_2-adrenoceptors.

There are three other aspects of the experimental conditions in our study that are worthy of comment. First, in preliminary experiments we observed that inhibition of neuronal uptake by cocaine, and blockade of β-adrenoceptors by propranolol, produced a 3 fold increase in the sensitivity of both preparations to noradrenaline (unpublished observations), and these agents were routinely incorporated in the Krebs-Henseleit saline. Secondly, although the inclusion of 10 μM corticosterone in the bathing medium further increased the sensitivity of the PCDA to noradrenaline, its failure to influence significantly either the slope of the Schild plot or pA_2 value for phentolamine (see below), coupled with a tendency to cause a slight reduction of the maximum response in both preparations, was taken as a basis for leaving extraneuronal uptake intact. Finally, no attempt was made to remove the endothelium, so the possibility exists that α-adrenoceptor-mediated contractions were reduced by either basal release or α_2-adrenoceptor-mediated release of endothelium-derived nitric oxide (Kovitz *et al.*, 1993; Ohgushi *et al.*, 1993). It should be noted, however, that endothelium-denudation in the PCDA failed to affect contractions to 5-hydroxytryptamine (Lot & Wilson, unpublished observations) and, α_2-adrenoceptor-mediated release of nitric oxide in the pig was not observed in mesenteric, renal or iliac arteries (Ohgushi *et al.*, 1993).

Noradrenaline produced concentration-dependent contractions in both preparations which were competitively antagonized by phentolamine. The pA_2 for phentolamine, approximately 7.3–7.7, is consistent with the presence of α-adrenoceptors (McGrath *et al.*, 1989). It was noticeable, however, that the potency of phentolamine was slightly higher in the PLV compared to the PCDA and that this difference was not influenced by the inclusion of 10 μM corticosterone in the bathing medium. The possibility exists that the decision to avoid full concentration-response curves in the PCDA, to reduce the potential for receptor desensitization, might have resulted in an underestimation of the potency of phentolamine in the arterial preparation.

To assess in greater detail the pharmacological characteristics of α-adrenoceptors in the two blood vessels, prazosin and YM-12617 were used as selective α_1-adrenoceptor antagonists and rauwolscine and CH-38083 used as selective α_2-adrenoceptor antagonists. Since, with the exception of prazosin (Akers *et al.*, 1987; Neilsen *et al.*, 1991), none of these agents has been used to characterize α-adrenoceptors on porcine vascular smooth muscle, no assumption was made about their selectivity for subtypes of α-adrenoceptor in the pig. Instead, we have relied upon agents of the same provisional class to produce qualitatively similar effects in each vessel. In this respect, the evidence suggests the presence of α_1- and α_2-adrenoceptors contributing to noradrenaline-induced contractions in the PCDA and PLV, with the latter possessing a larger population of functional α_2-adrenoceptors.

Prazosin and YM-12617 were potent antagonists in the PCDA but, unlike phentolamine, they produced non-competitive inhibition. Based upon the displacement produced by the lowest concentration employed (10 nM), the dissociation constant for both antagonists, approximately 9, is consistent with an effect at α_1-adrenoceptors (Wilson *et al.*, 1991). Significantly, higher concentrations of the antagonists tended to produce much smaller rightward displacement of

the lower component of the noradrenaline concentration-response curve, suggesting the presence of another receptor subtype. In the PCDA, only high concentrations of CH-38083 and rauwolscine (> 1 μM) produced significant inhibition of noradrenaline-induced responses which, when viewed in isolation, supports the presence of α_1-adrenoceptors. However, since the combination of a putative selective α_1- and α_2-adrenoceptor antagonist (prazosin and rauwolscine or YM-12617 and CH-38083) was more effective than the combination of either the putative α_1-adrenoceptor antagonists or α_2-adrenoceptor antagonists (prazosin with YM-12617 or CH-38083 and rauwolscine), this has been taken as evidence for the presence of α_2-adrenoceptors and also confirmation of the characteristics of the antagonists at α-adrenoceptors in the pig. Qualitatively similar observations were made in the PLV, except that the component of the noradrenaline response resistant to prazosin and YM-12617 was larger, and this was associated with a corresponding increase in the potency of rauwolscine and CH-38083; based upon the effect of the lowest concentration employed, rauwolscine and CH-38083 had an effect consistent with an action at α_2-adrenoceptors (see Wilson *et al.*, 1991). Finally, phenoxybenzamine, which has been reported to inactivate α_1-adrenoceptors preferentially (Constantine *et al.*, 1982), was more effective in the PCDA suggesting a larger population of α_1-adrenoceptors. Furthermore, following exposure to a combination of rauwolscine and phenoxybenzamine, to increase protection of α_2-adrenoceptors (Daly *et al.*, 1988c; Dunn *et al.*, 1991), the response to noradrenaline in PLV was completely resistant to prazosin but sensitive to rauwolscine; unequivocal evidence for a population of postjunctional α_2-adrenoceptors (McGrath *et al.*, 1989). In the case of the PCDA, however, only part of the remaining response was resistant to prazosin. The basis of this difference between the preparations is not known.

Although α_1- and α_2-adrenoceptors are currently divided into several further subtypes on the basis of both pharmacological characteristics of membrane sites in radioligand binding assays and receptor amino acid sequence (Wilson *et al.*, 1991; Lomasney *et al.*, 1991), the lack of genuinely subtype-specific antagonists, with selectivity comparable to that for either prazosin or rauwolscine, has precluded any attempt at detailed subclassification of α-adrenoceptors in these functional experiments. Moreover, since there is the potential for these receptor subtypes to interact (Daly *et al.*, 1988b,c; Dunn *et al.*, 1991), we have limited the scope of the pharmacological characterization simply to α_1- and α_2-adrenoceptors. It is noteworthy, however, that since we have used the resistance of responses to prazosin (0.1–1 μM) as a marker for the presence of postjunctional α_2-adrenoceptors, it seems likely that these belong to the $\alpha_{2A/D}$ subgroup. Also, the relative ease with which it is possible to obtain large quantities of porcine vascular smooth muscle has allowed us to perform preliminary radioligand binding experiments on the thoracic aorta and splenic artery (Wright *et al.*, 1993). Future experiments will be directed towards using membrane preparations of individual blood vessels for the purpose of detailed pharmacological characterization of the α-adrenoceptors.

Thus the PLV, like superficial veins from the rabbit, dog and man (Constantine *et al.*, 1982; Sjöberg *et al.*, 1987; Daly *et al.*, 1988a,b; Arner *et al.*, 1988) appear to possess α_1-adrenoceptors and a large population of α_2-adrenoceptors. Although α_1-adrenoceptors are the major subtype responsible for contractions to noradrenaline in the PCDA, observations with both competitive and irreversible antagonists indicate the presence of a small population of α_2-adrenoceptors which do not require the presence of an ancillary agent for functional expression (cf. Templeton *et al.*, 1989; Dunn *et al.*, 1991). In this respect, the PCDA is similar to both human skin and digital arteries (Stevens & Moulds, 1981; 1986; Borbujo *et al.*, 1989), and it provides support for the suggestion that the porcine blood vessels may be a good experimental model for man. Our results also suggest that demonstration of functional α_2-adrenoceptors on other human arteries, e.g. epigastric artery (Sjöberg *et al.*, 1987), may be more easily demonstrated by the use of noradrenaline and a combination of a selective α_1- and a selective α_2-adrenoceptor antagonist. It remains to be determined, however, whether the similarity between porcine and human α-adrenoceptor pharmacology in digital blood vessels extends to other vascular beds and, indeed, to other receptor systems, e.g. neuropeptidey and 5-HT_1-like, which have proved elusive in arterial vessels from standard laboratory animals.

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