The effectiveness of α_2 -adrenoceptor activation increases from the distal to the proximal part of the veins of canine limbs

Serafim Guimaraes & Jose P. Nunes

Department of Pharmacology, Faculty of Medicine, 4200-Porto, Portugal

- 1 The effectiveness of α_1 - and α_2 -adrenoceptor activation was compared at different levels of the saphenous and cephalic vein of the dog *in vitro*.
- 2 Helically cut strips were used to determine concentration-response curves to phenylephrine, noradrenaline, UK-14,304 (5-bromo-6-(imidazoline-2-ylamino)-quinoxaline) and B-HT 920 (2-amino-6-allyl-5,6,7,8-tetra-hydro-4H-(thiazo)-4,5-d-azepine). The effect of prazosin and yohimbine on these curves was also studied.
- 3 At the distal level, the maximum response to UK-14,304 amounted to 33 and 50% of those to noradrenaline in the saphenous and cephalic vein, respectively, while at the proximal level the maximum response to UK-14,304 amounted to 72 and 78% of those to noradrenaline, in the saphenous and cephalic vein, respectively.
- 4 In both vessels, the results obtained with B-HT 920 were very similar to those for UK-14,304.
- 5 The pD_2 values for UK-14,304 – which were identical at the three levels of both vessels – and the pA_2 values for the antagonism exerted by either prazosin or yohimbine against the responses to UK-14,304 indicate that the α_2 -adrenoceptors are identical at the different levels of both vessels.
- 6 These results show that the effectiveness of α_2 -adrenoceptor stimulation increases from the distal to the proximal regions of canine limb veins. Apparently, this is due to a greater density of α_2 -adrenoceptors in the proximal regions.
- 7 Yohimbine is much more potent against phenylephrine distally than proximally in both vessels. However, after 30 nM phenoxybenzamine – a concentration which eliminates the vast majority of α_1 -adrenoceptors without affecting α_2 -adrenoceptors – yohimbine became equally potent at both levels, suggesting that the difference existing before phenoxybenzamine depended on α_1 -adrenoceptors. Hence it is concluded that α_1 -adrenoceptors in distal and proximal portions may differ.

Introduction

The existence of two subgroups of postjunctional α -adrenoceptors on blood vessels was postulated on the basis of results obtained in experiments performed *in vivo* (Docherty *et al.*, 1979; Drew & Whiting, 1979; Timmermans *et al.*, 1979; McGrath *et al.*, 1982). In contrast to the ease with which confirmatory evidence for the existence of these two subgroups of α -adrenoceptors has been accumulating from experiments carried out *in vivo*, it is relatively difficult to demonstrate post-synaptic α_2 -adrenoceptors *in vitro*. It is now well established that although postjunctional α_2 -adrenoceptors are consistently found in many isolated veins of different species (De Mey & Vanhoutte, 1981; Shepperson & Langer, 1981; Constantine *et al.*, 1982; Shogi *et al.*, 1983; Guimaraes *et al.*, 1983; 1987) they are very scarce or simply are not detected in arteries (Starke & Docherty, 1982; Hamed *et al.*, 1983; Skärby & Andersson, 1984; Polonia *et al.*, 1985). However, there are exceptions to this (Flavahan *et al.*, 1987; Templeton *et al.*, 1989; Dunn *et al.*, 1989).

Recently, in perfusion experiments with distal segments of canine saphenous vein it was observed that they responded poorly or did not respond at all to α_2 -adrenoceptor agonists applied to either the intima or the adventitia (Nunes, De la Lande & Guimaraes, unpublished results). This unexpected finding prompted us to compare the effectiveness of α_2 -adrenoceptor activation at different levels of the canine saphenous vein.

Methods

Mongrel dogs, 12–20 kg in weight, of either sex, were anaesthetized with pentobarbitone sodium (30 mg kg⁻¹ injected in the forelimb) and segments of the saphenous and the cephalic vein trunks of about 25 mm in length were obtained from the

distal, intermediate or proximal parts of the veins (Figure 1). After removal of veins, the animals were killed by an overdose of pentobarbitone sodium (100 mg kg⁻¹). The distance between the three segments was of about 5 cm each. From each segment a pair of helically cut strips (of about 2.5 × 25 mm) were prepared and suspended in a 20 ml bath containing Krebs-Henseleit solution (Guimaraes & Osswald, 1969); the endothelium was removed by gentle rubbing of the intimal surface with paper moistened in Krebs solution. After this procedure, a ring of 0.1–0.2 mm length was removed from

Figure 1 (a) Lateral saphenous vein trunk of the right hindleg, lateral aspect. (b) Cephalic vein trunk of the right antebrachium, cranial aspect. The arrows indicate the sites from where the vein segments were removed.

each vein segment to confirm by microscopy the removal of the endothelium. The bath solution was kept at 37°C and bubbled with 95% O₂ and 5% CO₂. The strips were mounted between a stationary tissue holder and a transducer and isometric contractions were recorded as changes in grams of tension on a Harvard Universal Oscillograph.

Each strip was allowed to stabilize for 1 h under a basal tension of 2 g before experimentation. The bath solution was replaced several times during the equilibration period. Concentration-response curves to phenylephrine, noradrenaline, UK-14,304 (5-bromo-6-(imidazoline-2-ylamino)-quinoxaline) and B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-(thiazolo)-4,5-d-azepine) were determined by the method of stepwise cumulative additions of the agonist in the presence of cocaine (12 µM), to inhibit neuronal uptake (Trendelenburg, 1966), hydrocortisone (40 µM) to inhibit extraneuronal uptake (Iversen & Salt, 1970; Guimaraes *et al.*, 1978), and propranolol (1 µM) to block β -adrenoceptors (Black *et al.*, 1965).

The concentration of agonist in the bathing solution was increased 3 fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Two concentration-response curves were determined per strip. In experiments aimed at determining pD₂ values, two different agonists were used per strip, the order of administration being reversed in the paired strip; at the end of each experiment a maximum response was obtained to 300 µM noradrenaline, which was expressed in g g⁻¹ tissue. For the estimation of pA₂ values, two concentration-response curves were determined for one agonist per strip: one in the absence and the other in the presence of the antagonist, which was added 30 min before the determination of the second concentration-response curve. To detect changes in the sensitivity of the strips, time control concentration-response curves were determined for each agonist. Compensations were made whenever changes in sensitivity occurred with time. The use of high concentrations of UK-14,304 was accompanied by a quick development of desensitization (only to itself). Hence, only one concentration-response curve was determined for each of two paired strips: one in the presence and the other in the absence of the antagonist.

In some experiments, the effect of yohimbine was studied after the strips had been exposed to phenoxybenzamine (to eliminate α_1 -adrenoceptors). In these experiments, the strips were exposed to 30 or 100 nM phenoxybenzamine for 40 min and washed for 45 min before the first concentration-response curve was determined.

After completion of a concentration-response curve, drugs were washed out from the preparation. In all the experiments, propranolol, cocaine and hydrocortisone were added to the bath 30 min before the addition of the agonist and were left in the bath throughout the experiment.

In some experiments, nearly maximal responses to 150 mM potassium chloride (KCl) were also determined. This concentration was indicated by preliminary experiments in which 50, 100, 150 and 200 mM KCl were used. In the vast majority of these experiments, the lowest concentration eliciting the maximal response was 150 mM. No osmotic compensation was allowed for when KCl was used.

The pD₂ values represent the negative logarithm of the molar concentration of the agonist required to obtain 50% of the maximal effect of noradrenaline.

To study the antagonism by prazosin or yohimbine, pA₂ values were calculated according to the method of Van Rossum (1963) from the equation: $pA_2 = pA_x + \log(x - 1)$ in which x represents the factor of the shift of the concentration-response curve to the right and pA_x the negative logarithm of the molar concentration of the antagonist which caused this shift. As usually three different concentrations of each antagonist were used, slopes of Schild plots were calculated as well. To keep the selectivity of their antagonistic actions, both prazosin and yohimbine were used in concentrations as low as possible: for prazosin, 10⁻⁸, 3 × 10⁻⁸ and 10⁻⁷ M against

phenylephrine and 10⁻⁶, 3 × 10⁻⁶ and 10⁻⁵ M against UK-14,304, while for yohimbine the concentrations used were 10⁻⁸, 3 × 10⁻⁸ and 10⁻⁷ M against UK-14,304 and phenylephrine (distal vein), and 10⁻⁷, 3 × 10⁻⁷ and 10⁻⁶ M against phenylephrine (proximal vein).

The results are presented as arithmetic means with their standard errors (s.e.). Differences between means were compared by Student's *t* test. For multiple comparison between pairs of means, the Tukey-Kramer method was used (Sokal & Rohlf, 1981). A *P* value of 0.05 or less was considered significant.

Drugs

The drugs used were: B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-(thiazolo)-4,5-d-azepine) (Boehringer, Ingelheim, F.R.G.), cocaine hydrochloride (Uquipa, Lisboa, Portugal), hydrocortisone 21-phosphate (Sigma, St. Louis, MD, U.S.A.), (–)-noradrenaline bitartrate (Koch-Light, Haverhill, U.K.), phenoxybenzamine hydrochloride (Smith, Kline and French, Philadelphia, PA, U.S.A.), (–)-phenylephrine hydrochloride (Boehringer Sohn, Mannheim, F.R.G.), potassium chloride (Sigma), prazosin hydrochloride (Pfizer, Seixal, Portugal), (±)-propranolol hydrochloride (ICI, Macclesfield, U.K.), rauwolfscine hydrochloride (Roth, Karlsruhe, F.R.G.), UK-14,304 (5-bromo-6-(imidazoline)-2-ylamino)-quinoxaline (Pfizer), yohimbine hydrochloride (Sigma).

Results

Saphenous vein

Noradrenaline, phenylephrine, UK-14,304 and B-HT 920 caused concentration-dependent contractions of the strips obtained from distal, intermediate and proximal parts of the saphenous vein trunk. The maximal responses to noradrenaline and phenylephrine were identical at the three levels of the vein trunk (Figure 2 and Table 1). However, noradrenaline was approximately 15 times more potent than phenylephrine (Figure 2 and Table 2). Potassium chloride (KCl) (150 mM) and the selective α_2 -adrenoceptor agonists UK-14,304 and B-HT 920 caused maximal contractions which were smaller than those induced by noradrenaline. When expressed as % of the maximal response to noradrenaline, the maximal contraction to UK-14,304 reached 33.2 ± 3.7, 58.4 ± 3.7 and 72.1 ± 5.4% (*n* = 10), that to B-HT 920 27.5 ± 2.9, 51.6 ± 5.6 and 58.3 ± 6.3% (*n* = 5) and that to KCl 71.2 ± 7.6, 70.3 ± 6.1 and 73.6 ± 6.3% (*n* = 5) at the distal, intermediate and proximal levels, respectively (Figure 2 and Table 1).

Thus, maximal activation of the adrenoceptors with UK-14,304 and B-HT 920 results in markedly less contraction of the distal portion of the saphenous vein trunk than does

Table 1 Tension developed in saphenous vein with maximal contraction by different agonists

Agonist	Maximal contraction (in g g ⁻¹ tissue)			n
	Distal	Intermediate	Proximal	
Noradrenaline	304 ± 22	327 ± 20	318 ± 12	10
Phenylephrine	296 ± 16	339 ± 18	320 ± 35	5
UK-14,304	102 ± 6*	190 ± 12	228 ± 16	10
B-HT 920	82 ± 9*	168 ± 18	183 ± 20	5
KCl	215 ± 23	216 ± 19	232 ± 20	5

The mean weights of the strips were: 33, 38 and 40 mg (from the distal, intermediate and proximal regions, respectively).

* Significant difference between the value for the distal and that for the respective proximal portion, *P* < 0.05.

Figure 2 Concentration-response curves in dog saphenous vein to (a) noradrenaline, (b) phenylephrine, (c) UK-14,304 and (d) B-HT920 obtained at three different levels of the vessel: proximal (Δ), intermediate ($\blacksquare$) and distal ($\bullet$). Each point represents the mean and vertical lines show s.e.mean.

activation of adrenoceptors of the proximal portion of this vein trunk.

The effect of phenylephrine on the responses to UK-14,304 and vice versa was also checked. Neither sub-effective (up to 10 nM phenylephrine; up to 500 pM UK-14,304) nor effective

concentrations of one of these agonists enhanced or antagonized the responses to the other.

The pA_2 values for the antagonism exerted by prazosin and yohimbine on the effects of phenylephrine and UK-14,304 were determined only for the distal and the proximal segments

Table 2 pD_2 values for the contractile responses caused by different agonists on three different regions of the dog saphenous and cephalic vein trunks: distal, intermediate, and proximal (see Methods)

Agonist	pD_2			n
	Distal	Intermediate	Proximal	
Phenylephrine (Saphenous)	6.04 ± 0.04	6.26 ± 0.13	6.36 ± 0.06	5
	6.10 ± 0.07	6.21 ± 0.08	6.27 ± 0.05	4
Noradrenaline (Saphenous)	7.29 ± 0.04	7.39 ± 0.08	7.42 ± 0.07	5
	7.18 ± 0.03	7.21 ± 0.07	7.25 ± 0.06	4
UK-14,304 (Saphenous)	7.99 ± 0.10	8.10 ± 0.05	8.12 ± 0.01	7
	8.25 ± 0.03	8.20 ± 0.01	8.08 ± 0.06	4
B-HT 920 (Saphenous)	6.82 ± 0.05	6.75 ± 0.03	6.72 ± 0.06	5
	7.01 ± 0.09	6.97 ± 0.08	6.89 ± 0.07	3

$pD_2 = -\log EC_{50}$. Data shown are means $\pm$ s.e.

Table 3 pA_2 values and slopes of the Schild regressions for prazosin and yohimbine against the agonists phenylephrine and UK-14,304 in canine saphenous and cephalic veins

Antagonist	Agonist	Vein and segment used		pA_2	Slope	n
Prazosin	Phenylephrine	Distal	Saphenous	7.65 ± 0.07	0.99 ± 0.14	5
			Cephalic	7.62 ± 0.04	0.88 ± 0.21	4
		Proximal	Saphenous	7.40 ± 0.05	0.96 ± 0.23	5
			Cephalic	7.48 ± 0.06	1.08 ± 0.20	4
	UK-14,304	Distal	Saphenous	6.02 ± 0.06	1.03 ± 0.15	5
			Cephalic	5.98 ± 0.07	0.90 ± 0.17	4
		Proximal	Saphenous	5.93 ± 0.04	1.17 ± 0.17	5
			Cephalic	6.08 ± 0.05	1.06 ± 0.22	4
Yohimbine	Phenylephrine	Distal	Saphenous	8.14 ± 0.04	1.14 ± 0.14	4
			Cephalic	8.27 ± 0.18	1.06 ± 0.18	5
		Proximal	Saphenous	$6.90 \pm 0.05^*$	0.92 ± 0.20	7
			Cephalic	$7.17 \pm 0.16^*$	1.12 ± 0.13	5
	UK-14,304	Distal	Saphenous	8.79 ± 0.03	1.07 ± 0.19	5
			Cephalic	8.82 ± 0.03	1.19 ± 0.18	4
		Proximal	Saphenous	8.88 ± 0.01	0.94 ± 0.21	5
			Cephalic	8.93 ± 0.02	0.98 ± 0.18	4

* Significantly different from the value for the respective distal portion ($P < 0.01$; paired experiments). The slopes of Schild regressions were not significantly different from 1.

and are presented in Table 3. While the values for the antagonism exerted by yohimbine on the effects of UK-14,304 are similar at the distal and the proximal levels, those for the antagonism by yohimbine of phenylephrine are much higher at the distal than at the proximal level.

Since the selectivity of phenylephrine for α_1 -adrenoceptors is not very high (phenylephrine is about 30 times more effective on α_1 - than on α_2 -adrenoceptors; Starke, 1981) some involvement of α_2 -adrenoceptor activation may interfere with the pA_2 values for the antagonism of yohimbine against this amine. To avoid, as much as possible, α_2 -adrenoceptor stimulation by phenylephrine, incomplete concentration-response curves to this amine were determined such that the highest concentration of phenylephrine used after yohimbine was never higher than 30 times the minimal effective concentration before yohimbine (10 and 50 nM). The pA_2 values calculated under these conditions from the displacement of the concentration-response curve at the EC_{25} level, were 8.21 ± 0.18 ($n = 6$) at the distal and 7.36 ± 0.14 ($n = 6$) at the proximal levels ($P < 0.01$).

The pA_2 values for the antagonism by yohimbine of phenylephrine responses were also determined after 30 and 100 nM phenoxybenzamine. Very interestingly, after 40 min in 30 nM phenoxybenzamine, the maximum effect of phenylephrine reached $32.3 \pm 3.1\%$ ($n = 8$) of that obtained before phenoxybenzamine at distal and $60.2 \pm 5.4\%$ ($n = 8$) at proximal level, and the pD_2 values for phenylephrine were 5.21 ± 0.40 and 5.12 ± 0.51 ($n = 5$) at proximal and distal levels, respectively. Under the same conditions, the pA_2 values for yohimbine against phenylephrine were 8.47 ± 0.16 ($n = 6$) and 8.36 ± 0.14 ($n = 6$) at distal and proximal levels, respectively. After 100 nM phenoxybenzamine, it was impossible to compare pA_2 values, because at the distal level the response to phenylephrine was practically abolished in the majority of the strips; at the proximal level the maximum response to phenylephrine reached about 30% of that before phenoxybenzamine. The pA_2 value for prazosin vs UK-14,304 after phenoxybenzamine (30 nM) at proximal level was 6.01 ± 0.09 ($n = 6$).

The concentration-response curve for phenylephrine at distal level was displaced to the right by 10 and 30 nM rauwolscine (by factors of 2.5 ± 0.3 and 6.3 ± 0.7 , respectively; $n = 6$), while at proximal level, it was not changed. In the presence of 30 nM rauwolscine, yohimbine (30 nM) displaced the concentration-response curves for phenylephrine at proximal and distal levels similarly (by factors of 5.2 ± 0.6 and 5.4 ± 0.7 , respectively; $n = 6$) (Figure 3).

On vein strips of the same dogs, rauwolscine (30 nM) displaced the concentration-response curves for UK-14,304 to the right, equally at distal and proximal levels (by factors of 8.3 ± 1.1 and 9.5 ± 1.4 , respectively; $n = 6$) (Figure 3).

Cephalic vein

The results obtained in the cephalic vein are very similar to those obtained in the saphenous one. All the agonists caused concentration-dependent contractions of the strips obtained from distal, intermediate and proximal regions of the cephalic vein trunk. As shown in Figure 4, noradrenaline and phenylephrine caused maximal contractions of the same magnitude, while UK-14,304, B-HT 920 and KCl caused maximal contractions which were sub-maximal when compared to those caused by noradrenaline.

The maximal contractions to UK-14,304 reached about 50.2 ± 4.8 , 76.5 ± 8.1 and $78.1 \pm 6.7\%$ ($n = 10$) and those to B-HT 920 about 49.3 ± 5.1 , 76.4 ± 6.8 and $75.7 \pm 7.3\%$ ($n = 4$) of those to noradrenaline at the distal, intermediate and proximal portions, respectively, while the maximal contractions to KCl reached about 69% both at proximal and distal levels (Figure 4 and Table 4).

These results show that the maximal activation of postjunctional α_2 -adrenoceptors of the distal part of the cephalic vein generates a contraction that is much weaker than that induced by maximal activation of postjunctional α_2 -adrenoceptors of the proximal part of this vein trunk.

Table 4 Tension developed in cephalic vein with maximal contraction by different agonists

Agonist	Maximal contraction (in $g\ g^{-1}$ tissue)			n
	Distal	Intermediate	Proximal	
Noradrenaline	286 ± 28	357 ± 31	342 ± 32	10
Phenylephrine	282 ± 39	363 ± 36	347 ± 39	5
UK-14,304	$144 \pm 11^*$	272 ± 21	267 ± 28	10
B-HT 920	$139 \pm 18^*$	260 ± 27	258 ± 49	4
KCl	200 ± 21	—	239 ± 24	5

The mean weights of the strips were: 28, 33 and 36 mg (from the distal, intermediate and proximal regions, respectively).

* Significant difference between the value for the distal and that for the respective proximal portion.

Figure 3 Concentration-response curves for phenylephrine (a and c) and UK-14,304 (b and d) at proximal (a and b) and distal (c and d) levels, in the absence and presence of the α_2 -antagonists rauwolscine (Rauw) and yohimbine (Yoh). The responses to either agonist are expressed as % of its own maximum effect. In (a and c), (●) control, (▲) after 10 nM Rauw, (■) after 30 nM Rauw and (◆) after 30 nM Rauw + 30 nM Yoh; (b and d), (○) control, (□) after 30 nM Rauw. Each point represents the mean and vertical lines show s.e.mean.

Figure 4 Dog cephalic vein. Concentration-response curves to (a) noradrenaline, (b) phenylephrine, (c) UK-14,304 and (d) B-HT 920 obtained at three different levels of the vessel: proximal (Δ), intermediate ($\blacksquare$), and distal ($\bullet$). Each point represents the mean and vertical lines show s.e.mean.

The pA_2 values for the antagonism exerted by prazosin and yohimbine against the responses to phenylephrine and UK-14,304 were determined only for the distal and the proximal segments and are presented in Table 3. As in the saphenous vein, the pA_2 values for the antagonism by yohimbine of the effects of UK-14,304 are similar at the distal and proximal levels, while those for the antagonism by yohimbine of phenylephrine effects are higher at the distal than at the proximal level.

As for the saphenous vein, the pA_2 values for the antagonism exerted by yohimbine on phenylephrine responses after 30 nM phenoxybenzamine were not different at proximal and distal levels (8.39 ± 0.17 and 8.25 ± 0.12 , $n = 6$, respectively).

Discussion

It is known that in several vessels where α_1 - and α_2 -adrenoceptors co-exist postsynaptically, α_2 -adrenoceptor stimulation generates a low maximal response compared with α_1 -adrenoceptor stimulation. This has been shown for the canine saphenous (Flavahan *et al.*, 1984; Fowler *et al.*, 1984; Guimaraes, 1986) and cephalic vein (Moura *et al.*, 1990) as for other vessels of the dog and other species (Starke & Docherty, 1982; Polonia *et al.*, 1985; Fontes Ribeiro & Macedo, 1986). The present results obtained in the saphenous and the cephalic veins of the dog agree well with these data. In fact, the maximal response to the selective α_2 -adrenoceptor agonists UK-14,304 and B-HT 920 was smaller than the maximal responses to either noradrenaline or phenylephrine, thus allowing one to conclude that the maximal response to phenylephrine is most likely determined by α_1 -adrenoceptor activation.

The present results further show that in both the canine saphenous and cephalic veins, the tension per gram of tissue developed by the maximal responses to α_2 -adrenoceptor agonists increases from the distal to the proximal portions of those vessels, while those to noradrenaline, phenylephrine or KCl (activation not involving adrenoceptors) do not.

Interestingly, the maximal responses to phenylephrine, which were of the same magnitude at proximal and distal levels before phenoxybenzamine, became like those to α_2 -adrenoceptor agonists (larger at proximal than at distal level) after phenoxybenzamine. Bearing in mind that after phenoxybenzamine (30 nM) the responses to phenylephrine are predominantly α_2 -adrenoceptor-mediated (Guimaraes *et al.*, 1987), this finding clearly confirms that α_2 -adrenoceptor

stimulation is more effective at the proximal than at the distal level.

Since the maximal responses obtained in the intermediate portions of both the saphenous and cephalic veins are also intermediate between those obtained in the distal and proximal portions, one can conclude that the effectiveness of maximal α_2 -adrenoceptor activation increases from the distal to the proximal segments of those veins of the canine limbs.

The differences in the α_2 -adrenoceptor-mediated maximal responses observed in the present study may be the result of a number of factors: (1) a difference in the types of α_2 -adrenoceptors involved in the responses, (2) a different interaction between α_1 - and α_2 -adrenoceptors along the vessel, (3) a different α_2 -adrenoceptor density at the different levels, or (4) a difference in the α_2 -adrenoceptor-response coupling characteristics along the vessel. The first hypothesis can easily be ruled out. The pD_2 values for the selective α_2 -adrenoceptor agonists as well as the pA_2 describing interactions with selective α_2 -adrenoceptor antagonists did not change significantly from region to region in each vessel, showing that receptors involved in responses to UK-14,304 and B-HT 920 are identical along the vessel. The same arguments are valid against the second hypothesis. Moreover, if there were a different interaction between α_1 - and α_2 -adrenoceptors along the vessel, the combination of α_1 and α_2 -adrenoceptor agonists should be synergistic or antagonistic at one or other level and not additive along all the vessel, as was the case. Thus, the increase in the maximal response to α_2 -adrenoceptor agonists in proximal compared with distal portions of both veins cannot be ascribed to some difference in the α_2 -adrenoceptors involved or to a different interaction between α_1 - and α_2 -adrenoceptors.

Since the receptors may vary with respect to their density in various types of tissues, both at the central and peripheral levels (Timmermans & Van Zwieten, 1981), it is likely that the differences in the α_2 -adrenoceptor-mediated maximal responses observed in the present study result from a difference in α_2 -adrenoceptor density at the different levels. The abolition of the responses to phenylephrine at the distal level after 100 nM phenoxybenzamine, while a 30% maximal response still remained at the distal one, is highly suggestive of a lower density of α -adrenoceptors at the distal level. However, some difference in the receptor-response coupling characteristics along the vessel cannot be excluded as a causal factor.

In the last few years, evidence has accumulated in favour of the view that α_1 -adrenoceptors do not have the same properties in all tissues. Analysis of α -adrenoceptor antagonists'

potencies in isolated vessels suggests that postjunctional α_1 -adrenoceptors are heterogeneous (Flavahan & Vanhoutte, 1986; Minneman, 1988). In our experiments, which were carried out after inhibition of the different processes which remove the agonists from the α -adrenoceptor biophase, yohimbine was much more effective against the responses to phenylephrine at the distal than at the proximal level. The pA_2 values (8.14 at distal and 6.90 at proximal level in the saphenous, and 8.24 at distal and 7.17 at proximal level in the cephalic vein) show that yohimbine is 17 and 13 times more potent distally than proximally (in the saphenous and cephalic vein, respectively). Although the presence of α_2 -adrenoceptors can disturb the analysis of α_1 -adrenoceptor-mediated responses and vice versa in tissues where both subgroups coexist (McGrath *et al.*, 1989), these differences can hardly be explained on the basis of a different involvement of α_2 -adrenoceptor activation at these two levels. In favour of the view that α_2 -adrenoceptors are more involved in the responses to phenylephrine at the distal than at the proximal level – thus explaining the differential antagonism by yohimbine – it might be argued that in the presence of low concentrations of rauwolscine, yohimbine became equally potent against phenylephrine at both levels. But who knows what rauwolscine did in low concentrations? Did it antagonize classical α_2 -adrenoceptors whose role is more prominent at the distal level or did it antagonize 'special' α_1 -adrenoceptors existing at the distal level which are more sensitive to α_2 -adrenoceptor antagonists than are the classical α_1 -adrenoceptors? All the evidence obtained in the present investigation is in favour of the second hypothesis: firstly, if α_2 -adrenoceptor activation had any involvement in this differential effect of yohimbine, yohimbine should have antagonized phenylephrine more effectively at the proximal than at the distal level – since α_2 -adrenoceptor activation is more effective proximally than distally – and not the opposite; furthermore, UK-14,304, which is a highly selective α_2 -adrenoceptor agonist, was equally antagonized at the distal and the proximal level by either yohimbine and rauwolscine. If phenylephrine acted on the same receptors, why wasn't it antagonized equally by these antagonists at those levels? Additionally, in the experiments carried out under conditions which did not favour α_2 -adrenoceptor stimulation by phenylephrine – those in which incomplete concentration-response curves to phenylephrine were determined – yohimbine continued to be much more potent at distal than at proximal level, showing that the differential effect of yohimbine only depends on the α_1 -adrenoceptor population. Some years ago, several authors

using different experimental procedures, showed that in low concentrations, phenoxybenzamine inactivates α_1 -adrenoceptors only: in the cat's spleen, Dubocovich & Langer (1974) demonstrated that the concentration of phenoxybenzamine required for the blockade of presynaptic α_2 -adrenoceptors that regulate transmitter release is 30 times higher than that required for the blockade of postsynaptic α_1 -adrenoceptors; in the pithed rat, Ruffolo *et al.* (1984) showed that 1 mg kg^{-1} phenoxybenzamine will irreversibly inactivate approximately 84% of the α_1 -adrenoceptors without affecting α_2 -adrenoceptors; in the canine saphenous vein, one of the tissues used in the present study, it was shown that 30 nM phenoxybenzamine – a concentration which did not affect α_2 -adrenoceptors – reduced the α_1 -adrenoceptor population such that the responses to phenylephrine become predominantly α_2 -adrenoceptor-mediated (Guimaraes *et al.*, 1987). With these data in mind, the antagonism of yohimbine against phenylephrine was also checked after 30 nM phenoxybenzamine, a concentration which eliminates the vast majority of α_1 -adrenoceptors without affecting α_2 -adrenoceptors. Under these conditions, yohimbine became equally potent at the proximal and the distal levels, showing that elimination of α_1 -adrenoceptors removes the source for that differential antagonism by yohimbine. Hence, the difference in sensitivity to yohimbine of the responses to phenylephrine at the two levels may be due to a difference in the α_1 -adrenoceptors involved.

In 1982, Ruffolo *et al.* demonstrated that in the rat aorta, α -adrenoceptors possess properties of both α_1 - and α_2 -adrenoceptors, since both prazosin and yohimbine are very potent against phenylephrine in this tissue. The α -adrenoceptors mediating the responses to phenylephrine in the distal portions of the veins of canine limbs seem to be like those described by these authors.

In summary, the present results suggest changes in both postjunctional α_1 - and α_2 -adrenoceptors along the canine saphenous and cephalic vein trunks: while α_2 -adrenoceptors increase in density from the distal towards the proximal portion, α_1 -adrenoceptors in distal and proximal portions may be of a different type.

The authors are deeply indebted to the Humboldt-Stiftung (Bonn, F.R.G.) for generously providing some of the material. The authors wish also to thank Dr Goulden, the Pfizer Company, for the generous gift of UK-14,304.

This work was supported by INIC (Instituto Nacional de Investigação científica) (FMP1).

References

- BLACK, J.W., DUNCAN, W.A.M. & SHANKS, R.G. (1965). Comparison of some properties of pronethanol and propranolol. *Br. J. Pharmacol.*, **25**, 577–591.
- CONSTANTINE, J.W., LEBEL, W. & ARCHER, R. (1982). Functional postsynaptic α_2 - but not α_1 -adrenoceptors in dog saphenous vein exposed to phenoxybenzamine. *Eur. J. Pharmacol.*, **85**, 325–329.
- DE MEY, J.C. & VANHOUTTE, P.M. (1981). Uneven distribution of postjunctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. *Circ. Res.*, **48**, 875–884.
- DOCHERTY, J.R., MACDONALD, A. & McGRATH, J.C. (1979). Further sub-classification of α -adrenoceptors in the cardiovascular system, vas deferens and anococcygeus of the rat. *Br. J. Pharmacol.*, **67**, 421P–422P.
- DREW, G.M. & WHITING, S.B. (1979). Evidence for two distinct types of postsynaptic α -adrenoceptor in vascular smooth muscle *in vivo*. *Br. J. Pharmacol.*, **67**, 207–215.
- DUBOCOVICH, M.L. & LANGER, S.Z. (1974). Negative feedback regulation of noradrenaline release by nerve stimulation in the perfused cat's spleen: differences in potency of phenoxybenzamine in blocking the pre- and post-synaptic receptors. *J. Physiol.*, **237**, 505–519.
- DUNN, W.R., McGRATH, J.C. & WILSON, V.G. (1989). Expression of functional postjunctional α_2 -adrenoceptors in rabbit isolated distal saphenous artery – a permissive role for angiotensin II? *Br. J. Pharmacol.*, **96**, 259–261.
- FLAVAHAN, N.A. & VANHOUTTE, P.M. (1986). α_1 -Adrenoceptor sub-classification in vascular smooth muscle. *Trends Pharmacol. Sci.*, **7**, 347–349.
- FLAVAHAN, N.A., RIMELE, T.J., COOKE, J.P. & VANHOUTTE, P.M. (1984). Characterization of postjunctional α_1 - and α_2 -adrenoceptors activated by exogenous or nerve released norepinephrine in the canine saphenous vein. *J. Pharmacol. Exp. Ther.*, **230**, 699–705.
- FLAVAHAN, N.A., COOKE, J.P., SHEPHERD, J.T. & VANHOUTTE, P.M. (1987). Human postjunctional α_1 - and α_2 -adrenoceptors: differential distribution in arteries of the limbs. *J. Pharmacol. Exp. Ther.*, **241**, 361–365.
- FONTES RIBEIRO, C.A. & MACEDO, T.A. (1986). Pharmacological characterization of the postsynaptic α -adrenoceptors in human uterine artery. *J. Pharm. Pharmacol.*, **38**, 600–605.
- FOWLER, P.J., GROUS, M., PRICE, W. & MATTHEWS, W.D. (1984). Pharmacological differentiation of postsynaptic α adrenoceptors in the dog saphenous vein. *J. Pharmacol. Exp. Ther.*, **229**, 712–718.
- GUIMARAES, S. (1986). Postsynaptic α -adrenoceptors in blood vessels: discrepancies between results obtained *in vivo* and *in vitro*. In *New Aspects of the Role of Adrenoceptors in the Cardiovascular System*, ed. Grobecker, H., Philippu, A. & Starke, K., pp. 129–138. Berlin: Springer-Verlag.

- GUIMARAES, S., BRANDAO, F. & PAIVA, M.Q. (1978). A study of the adrenoceptor-mediated feedback mechanism by using adrenaline as a false transmitter. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **305**, 185–188.
- GUIMARAES, S. & OSSWALD, W. (1969). Adrenergic receptors in the veins of the dog. *Eur. J. Pharmacol.*, **5**, 133–140.
- GUIMARAES, S., PAIVA, M.Q. & POLONIA, J.J. (1983). Alpha₁ and alpha₂-adrenoceptors of the dog saphenous vein and their relation to the sympathetic nerve terminals. *Progr. Neuropsychopharmacol.* (Abstracts) (Suppl.), 155.
- GUIMARAES, S., PAIVA, M.Q. & MOURA, D. (1987). Alpha₂-adrenoceptor-mediated responses to so-called selective alpha₁-adrenoceptor agonists after partial blockade of alpha₁-adrenoceptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **335**, 397–402.
- HAMED, A.T., JOHNSON, T.D., CHARLTON, K.G. & CLARKE, D.E. (1983). Pharmacological characterization of α -adrenoceptor subtypes in rat isolated thoracic aorta. *J. Auton. Pharmacol.*, **3**, 265–273.
- IVERSEN, L.L. & SALT, P.J. (1970). Inhibition of catecholamine uptake₂ by steroids in the isolated rat heart. *Br. J. Pharmacol.*, **40**, 528–530.
- MCGRATH, J.C., BROWN, C.M. & WILSON, V.G. (1989). Alpha-adrenoceptors: A critical review. *Med. Res. Rev.*, **9**, 407–533.
- MCGRATH, J.C., FLAVAHAN, N.A. & MCKEAN, C.E. (1982). α_1 - and α_2 -adrenoceptor mediated pressor and chronotropic effects in rat and rabbit. *J. Cardiovasc. Pharmacol.*, **4**, S101–S107.
- MINNEMAN, K.P. (1988). α_1 -Adrenergic receptor subtypes, inositol phosphates, and sources of cell Ca²⁺. *Pharmacol. Rev.*, **40**, 87–119.
- MOURA, D.M., VAZ-DA-SILVA, M.J. & GUIMARAES, S. (1990). Alpha-adrenoceptor reserve in the canine cephalic vein. *Eur. J. Pharmacol.*, **179**, 9–16.
- POLONIA, J.J., PAIVA, M.Q. & GUIMARAES, S. (1985). Pharmacological characterization of postsynaptic α -adrenoceptor subtypes in five different dog arteries *in vitro*. *J. Pharm. Pharmacol.*, **37**, 205–208.
- RUFFOLO, R.R., Jr., MORGAN, E.L. & MESSICK, K. (1984). Possible relationship between receptor reserve and the differential antagonism of alpha₁- and alpha₂-adrenoceptor-mediated pressor responses by calcium channel antagonists in the pithed rat. *J. Pharmacol. Exp. Ther.*, **230**, 587–594.
- RUFFOLO, R.R., Jr., WADDELL, J.E. & YADEN, E.C. (1982). Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. *J. Pharmacol. Exp. Ther.*, **221**, 309–314.
- SHEPPERSON, N.B. & LANGER, S.Z. (1981). The effects of the 2-amino-tetrahydronaphthalene derivative M7 a selective α_2 -adrenoceptor agonist *in vitro*. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **318**, 10–13.
- SHOJI, T., TSURU, H. & SHIGEI, T. (1983). A regional difference in the distribution of postsynaptic alpha-adrenoceptor subtypes in canine veins. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **324**, 246–255.
- SKARBY, T. & ANDERSSON, K.-E. (1984). Contraction-mediated α -adrenoceptors in isolated human omental, temporal and pial arteries. *J. Auton. Pharmacol.*, **4**, 219–229.
- SOKAL, R.R. & ROHLF, F.J. (1981). *Biometry. The Principles and Practice of Statistics in Biological Research*. New York: Freeman and Company.
- STARKE, K. (1981). α -Adrenoceptor sub-classification. *Rev. Physiol. Biochem. Pharmacol.*, **88**, 199–236.
- STARKE, K. & DOCHERTY, J.R. (1982). Types and functions of peripheral α -adrenoceptors. *J. Cardiovasc. Pharmacol.*, **4**, S3–S7.
- TEMPLETON, A.G.B., MACMILLAN, J., MCGRATH, J.C., STOREY, N.D. & WILSON, V.G. (1989). Evidence for prazosin-resistant, rauwolscine-sensitive α -adrenoceptors mediating contractions in the isolated vascular bed of the rat tail. *Br. J. Pharmacol.*, **97**, 563–571.
- TIMMERMANS, P.B.M.W.M., KWA, H.Y. & VAN ZWIETEN, P.A. (1979). Possible subdivision of postsynaptic α -adrenoceptors mediating pressor response in the pithed rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **310**, 189–193.
- TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1981). The postsynaptic α_2 -adrenoceptor. *J. Auton. Pharmacol.*, **1**, 171–183.
- TRENDELENBERG, U. (1966). Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. *Pharmacol. Rev.*, **18**, 629–640.
- VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.*, **143**, 299–330.

(Received February 16, 1990

Revised May 25, 1990

Accepted June 1, 1990)