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# Postsynaptic α-adrenoceptor reserve and the shift of the concentration-response curves to the right, as caused by the irreversible α-adrenoceptor antagonist phenoxybenzamine

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**1** The effect of different concentrations of phenoxybenzamine (0.1, 0.3, 1, 3, 10 and 30 nmol $l^{-1}$) on the concentration-response curves to phenylephrine (a selective $\alpha_1$-adrenoceptor agonist) and noradrenaline (a mixed $\alpha_1$- and $\alpha_2$-adrenoceptor agonist) was compared in two kinds of vascular tissue: dog saphenous vein (has both postsynaptic $\alpha_1$- and $\alpha_2$-adrenoceptors) and dog mesenteric and renal arteries – where only postsynaptic $\alpha_1$-adrenoceptors have been shown to exist.

**2** In the saphenous vein, where both $\alpha_1$- and $\alpha_2$-adrenoceptors coexist, at only one concentration of phenoxybenzamine, 3 nmol $l^{-1}$, the concentration-response curve of noradrenaline was shifted to the right without a reduction of the maximum; and this shift was small (by 0.4 log units).

**3** In tissues where only $\alpha_1$-adrenoceptors exist postsynaptically (mesenteric and renal arteries) phenoxybenzamine never caused any shift of the noradrenaline concentration-response curves to the right without depressing the maximum effect.

**4** In none of the tissues did phenoxybenzamine at any concentration shift the concentration-response curve of phenylephrine to the right without depressing its maximum.

**5** All these results indicate that in the dog saphenous vein there is a 'false' α-adrenoceptor reserve for noradrenaline, since two kinds of receptors participate in the response to this amine.

**6** The calculation of the occupancy-response relationship for the renal artery showed that 24% of the maximal response occurs when only 2% of $\alpha_1$-adrenoceptors are activated and 50% of maximum at 9% occupation. However, for 95% of the maximal response an 83% occupancy is required. Similar values were calculated for the mesenteric artery.

**7** Thus, the surplus $\alpha_1$-adrenoceptors which is very large for a half-maximal response becomes smaller and smaller as the magnitude of the response increases and probably disappears at the 100% response level.

**8** If we retain the original definition of 'spare receptors' – receptors in 'excess' of those required to produce a maximal response, we conclude, that there is no receptor reserve in the dog mesenteric and renal arteries.

## Introduction

The concept of spare α-adrenoceptors gained new importance when it was suggested that differential antagonism by calcium entry blockers of the responses mediated by vascular $\alpha_1$- and $\alpha_2$-adrenoceptors could be shown. This was related to differences in receptor reserves between the postsynaptic vascular α-adrenoceptor subtypes rather than a specific blockade of the processes set in motion by activation of the receptor subtypes (Hamilton *et al.*, 1983; Ruffolo & Yaden, 1984; Ruffolo *et al.*, 1984).

The evidence supporting this hypothesis is that after irreversible blockade of about 80% of postsynaptic vascular $\alpha_1$-adrenoceptors by low doses of phenoxybenzamine, the responses to a selective $\alpha_1$-adrenoceptor agonist became as sensitive to antagonism by calcium entry blockers as $\alpha_2$-adrenoceptor-mediated responses (Ruffolo *et al.*, 1984).

However, it was demonstrated in the dog saphenous vein *in vitro* that after low concentrations of phenoxybenzamine the effects caused by selective $\alpha_1$-adren-

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oceptor agonists are predominantly $\alpha_2$-adrenoceptor-mediated (Guimarães *et al.*, 1987). Thus, after phenoxybenzamine, calcium entry blockers might equally antagonize responses evoked by $\alpha_1$- and $\alpha_2$-adrenoceptor agonists because all responses are then mediated by $\alpha_2$-adrenoceptors and these are indeed differentially susceptible.

On the other hand, the existence of a receptor reserve is a matter of much controversy (Sastre & Milnor, 1985; Kenakin, 1986) and considered not to be supported by experimental evidence in the case of responses to sympathomimetic agonists (Furchgott, 1972).

Bearing all these facts in mind, we decided to examine the presence of a receptor reserve in three different vascular tissues *in vitro*. In this study we compared the effect of different concentrations of phenoxybenzamine (0.1, 0.3, 1, 3, 10 and 30 nmol $l^{-1}$) on the concentration-response curves of phenylephrine (a selective $\alpha_1$-adrenoceptor agonist; Starke *et al.*, 1975) and noradrenaline (a mixed $\alpha_1$ and $\alpha_2$-adrenoceptor agonist; Starke, 1981) in two kinds of vascular tissue; dog saphenous vein, which has both postsynaptic $\alpha_1$- and $\alpha_2$-adrenoceptors (De Mey & Vanhoutte, 1980; Polónia *et al.*, 1985), and dog mesenteric and renal arteries, where only $\alpha_1$-adrenoceptors exist postsynaptically (Polónia *et al.*, 1985).

## Methods

Mongrel dogs, 8–14 kg in weight, of either sex, were anaesthetized with pentobarbitone sodium (30 mg $kg^{-1}$ i.v. injected in the forelimb) and segments of cranial mesenteric and renal arteries and of saphenous vein were removed.

Helically cut strips of about 2.5 × 30 mm were prepared and suspended in a 20 ml bath containing Krebs-Henseleit solution (Guimarães & Osswald, 1969) after the endothelium had been removed by gentle rubbing of the intimal surface with filter paper moistened in the Krebs solution. The bath solution was kept at 37°C and bubbled with 95% $O_2$ and 5% $CO_2$. The strips were connected to isotonic levers and adjusted to give an approximately 5 fold magnification and counterweighed to provide a load of 2 g. The responses were recorded with a frontal lever on a smoked drum. Each strip was allowed to stabilize during an equilibration period of 1 h.

Concentration-response curves to phenylephrine and noradrenaline were determined by the method of stepwise cumulative addition of the agonist in the presence of cocaine (12 μmol $l^{-1}$) to inhibit neuronal uptake (Trendelenburg, 1966), hydrocortisone (40 μmol $l^{-1}$) to inhibit extraneuronal uptake (Iversen & Salt, 1970; Guimarães *et al.*, 1978) and propranolol (1 μmol $l^{-1}$) to block β-adrenoceptors (Black *et al.*, 1965).

The concentration of the 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. After completion of a concentration-response curve, drugs were washed out from the preparation.

Propranolol and cocaine were added to the bath 30 min before the addition of the agonist and they were left in the bath throughout the experiment.

After wash-out the strips were exposed to phenoxybenzamine (0.1, 0.3, 1, 3, 10 or 30 nmol $l^{-1}$) for 30 min and washed for 45 min before determining the second concentration-response curve.

In some experiments phenoxybenzamine + yohimbine (20 or 40 nmol $l^{-1}$) was used. In these experiments yohimbine was re-added 30 min before determining the second concentration-response curve.

### *Determination of dissociation constants*

The dissociation constants ($K_A$) of phenylephrine were determined by the method of Furchgott & Bursztyn (1967). Concentration-response curves to phenylephrine were obtained before and 30 min after partial irreversible α-adrenoceptor inactivation by phenoxybenzamine. Treatment with phenoxybenzamine (1 and 3 nmol $l^{-1}$ for the mesenteric and renal arteries, respectively) shifted the respective concentration-response curves of phenylephrine to the right in a non-parallel manner and depressed the maximum response to about 35%.

Plots of the reciprocals of phenylephrine concentrations before fractional inactivation against the reciprocals of the corresponding equieffective concentrations of phenylephrine after receptor inactivation yielded straight lines in accord with receptor theory (Furchgott, 1972). The $K_A$ of phenylephrine was then calculated from the slope and intercept of the resulting 'double reciprocal' plots by the following equation (Furchgott & Bursztyn, 1967):

$$K_A = \frac{\text{slope} - 1}{\text{intercept}}$$

### *Calculation of α-adrenoceptor occupancy-response relationships*

Fractional α-adrenoceptor occupancy by phenylephrine was calculated for each bath concentration studied ([A]) using the dissociation constant ($K_A$). The following relationship between agonist concentration ([A]) and dissociation constant was used to calculate α-adrenoceptor occupancy by the agonist:

$$\%\ \text{receptor occupancy} = \frac{[A]}{K_A + [A]} \times 100$$

*Drugs*

The drugs used were: cocaine hydrochloride (Uquipa, Lisboa, Portugal), (−)-noradrenaline bitartrate (Koch-Light, Haverhill, UK), (−)-phenylephrine hydrochloride (Boehringer Sohn, Mannheim, R.F.G.), phenoxybenzamine hydrochloride (Smith Kline and French, Philadelphia, U.S.A.), (±)-propranolol hydrochloride (ICI, Cheshire, U.K.), yohimbine hydrochloride (Sigma, St. Louis, U.S.A.)

*Statistical analysis*

Responses to agonists are expressed as % of the maximum response. Maximum responses to noradrenaline and phenylephrine were not significantly different in any preparation.

The results are presented as arithmetic means with their standard errors (s.e.) or as geometric means with 95% confidence limits. Differences between means were compared by Student's $t$ test and those with $P$ values of 0.05 or less were considered significant.

## Results

*Saphenous vein*

Concentration-response curves were determined for noradrenaline and phenylephrine. The maximal response caused by phenylephrine was of about the same magnitude as that produced by noradrenaline, as previously demonstrated by Guimarães (1975). As shown before (Guimarães, 1975), noradrenaline is much more potent than phenylephrine in this tissue (Figure 1). The concentration-response curve for phenylephrine was steeper than that for noradrenaline (Figure 1 and Table 1).

As shown in Figure 1, 1 nmol $l^{-1}$ phenoxybenzamine did not modify the concentration-response curves for either noradrenaline or phenylephrine (Figure 1a). At 3 nmol $l^{-1}$, phenoxybenzamine caused significant displacements to the right of these curves: without depression of the maximum in the case of noradrenaline; with about 10% reduction of the maximum in the case of phenylephrine (Figure 1b). At 10 nmol $l^{-1}$, phenoxybenzamine shifted the concentration-response curves for noradrenaline and phenylephrine to the right and depressed the maximum in both cases: the depression of the concentration-response curve for phenylephrine was more pronounced (by 18.1 ± 2.8%; $n = 5$) than that of the concentration-response curve for noradrenaline (by 9.2 ± 1.6%; $n = 7$) (Figure 1c). At the highest concentration used (30 nmol $l^{-1}$), phenoxybenzamine shifted the concentration-response curves of both phenylephrine and noradrenaline to the right and depressed their maximal values (by 31 ± 2.8%; $n = 6$ and by 21 ± 3.2%; $n = 7$ respectively) (Figure 1d).

*Mesenteric artery*

Noradrenaline and phenylephrine produced maximal responses of about the same magnitude. As previously found (Guimarães & Paiva, 1977), noradrenaline was more potent than phenylephrine (Figure 2).

The slopes of the concentration-response curves for noradrenaline and phenylephrine were not different but for noradrenaline it was steeper than in the saphenous vein (Table 1).

Phenoxybenzamine was used in concentrations lower than those used in the saphenous vein, because results obtained in preliminary experiments indicated that it was about 10 times more effective in the mesenteric artery than in the saphenous vein.

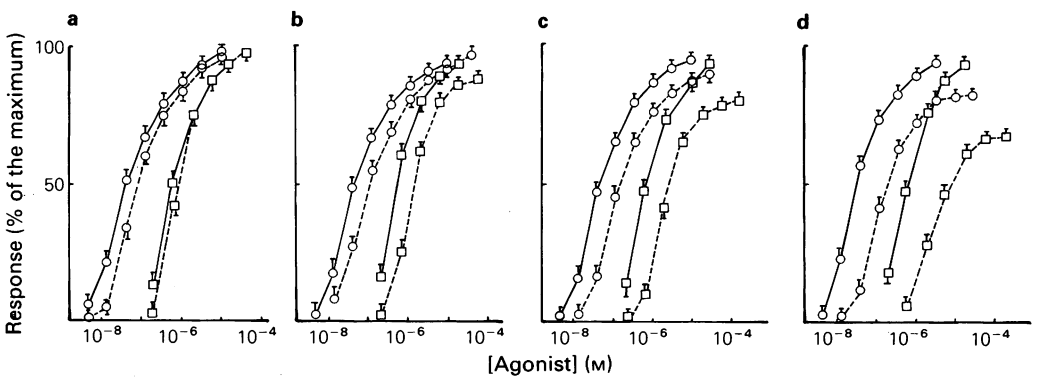


**Figure 1** Saphenous vein of the dog. Concentration-response curves to noradrenaline (○) and phenylephrine (□) determined in the presence of cocaine (12 μmol $l^{-1}$), hydrocortisone (40 μmol $l^{-1}$) and propranolol (1 μmol $l^{-1}$) before (unbroken lines) and after (broken lines) phenoxybenzamine: 1 nmol $l^{-1}$, in (a); 3 nmol $l^{-1}$, in (b); 10 nmol $l^{-1}$, in (c); 30 nmol $l^{-1}$, in (d).

**Table 1** The slopes of the concentration-response curves of noradrenaline and phenylephrine calculated from the points corresponding to probits 4 and 6 (16% and 86%).

| | *Saphenous vein* | n | *Mesenteric artery* | n | *Renal artery* | n |
|---|---|---|---|---|---|---|
| Noradrenaline | $1.01 \pm 0.04^a$ | 10 | $1.79 \pm 0.10^b$ | 12 | $0.98 \pm 0.05^c$ | 12 |
| Phenylephrine | $1.58 \pm 0.06^d$ | 10 | $1.80 \pm 0.10^e$ | 12 | $1.07 \pm 0.08^f$ | 12 |

The differences were significant between a and b; a and d; d and f; e and f.

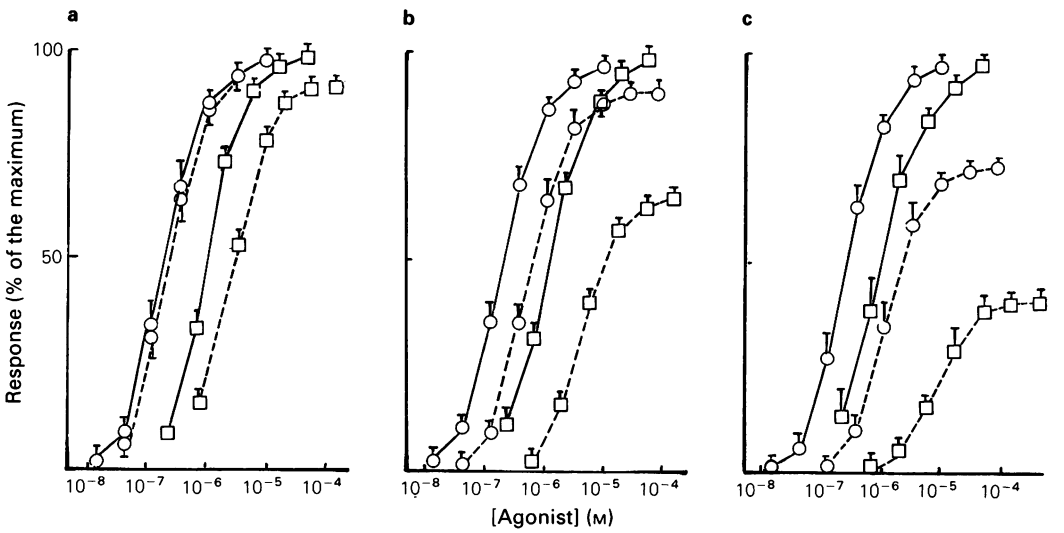


**Figure 2** Mesenteric artery of the dog. Concentration-response curves to noradrenaline (○) and phenylephrine (□) determined in the presence of cocaine (12 $\mu$mol $l^{-1}$), hydrocortisone (40 $\mu$mol $l^{-1}$) and propranolol (1 $\mu$mol $l^{-1}$) before (unbroken lines) and after (broken lines) phenoxybenzamine: 0.3 nmol $l^{-1}$, in (a); 1 nmol $l^{-1}$, in (b); 3 nmol $l^{-1}$, in (c).

At a concentration of 0.3 nmol $l^{-1}$, phenoxybenzamine did not cause any change of the concentration-response curve for noradrenaline but it displaced to the right that for phenylephrine and caused a depression of its maximum by $9.0 \pm 2.5\%$ ($n = 5$) (Figure 2a). At a concentration of 1 nmol $l^{-1}$, phenoxybenzamine shifted to the right the concentration-response curves for both noradrenaline and phenylephrine and depressed their maximal values: more for phenylephrine than for noradrenaline (by $35.1 \pm 8.1$ and $9.5 \pm 2.5\%$, respectively; $n = 11$; $P < 0.01$) (Figure 2b). At the highest concentration used (3 nmol $l^{-1}$) phenoxybenzamine caused displacements of both concentration-response curves to the right. These shifts were larger than those caused by the lower concentrations and were accompanied by depressions of the maximal values which were also larger than those caused by 1 nmol $l^{-1}$ phenoxybenzamine: $58.9 \pm 5.8\%$ for phenylephrine and $25.7 \pm 5.7\%$ for noradrenaline ($n = 7$; $P < 0.01$) (Figure 2c).

In the presence of 20 nmol $l^{-1}$ yohimbine the depressions of the maximum caused by 3 and 10 nmol $l^{-1}$ phenoxybenzamine on the concentration-response curves of noradrenaline and phenylephrine were still more marked for phenylephrine than for noradrenaline, although the difference tended to be smaller than between depressions obtained in the absence of yohimbine (not shown).

*Renal artery*

In the renal artery, the concentration-response curves for phenylephrine and noradrenaline had similar slopes, which were flatter than those obtained in the mesenteric artery, and their maximal values were of about the same magnitude (Figure 3). As previously found (Polónia *et al.*, 1985), the potency of noradrenaline was higher than that of phenylephrine (Figure 3).

At the lowest concentration used (0.1 nmol $l^{-1}$) phenoxybenzamine did not cause any significant

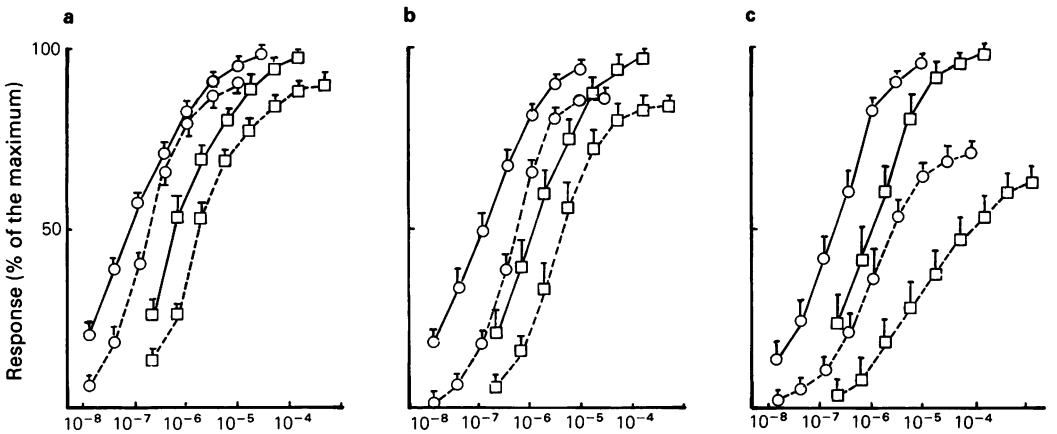


**Figure 3** Renal artery of the dog. Concentration-response curves to noradrenaline (○) and phenylephrine (□) determined in the presence of cocaine (12 μmol $l^{-1}$), hydrocortisone (40 μmol $l^{-1}$) and propranolol (1 μmol $l^{-1}$) before (unbroken lines) and after (broken lines) phenoxybenzamine: 0.3 nmol $l^{-1}$, in (a); 1 nmol $l^{-1}$, in (b); 3 nmol $l^{-1}$, in (c).

change of either concentration-response curve. As shown in Figure 3a, at a three times higher concentration (0.3 nmol $l^{-1}$), phenoxybenzamine caused a displacement of the concentration-response curves for both noradrenaline and phenylephrine to the right and reduced their maximal values (by 9.1 ± 2.6% and 11.0 ± 3.2%; $n = 4$, respectively). At a concentration of 1 nmol $l^{-1}$, phenoxybenzamine displaced the concentration-response curves for noradrenaline and phenylephrine to the right and caused larger reductions of their maximal values (by 18.8 ± 4.3 and 16.7 ± 3.0%; $n = 6$, respectively) (Figure 3b). At the highest concentration used (3 nmol $l^{-1}$), phenoxybenzamine shifted both concentration-response curves to the right and caused reductions of their maximal values which were larger than those caused by 1 nmol $l^{-1}$ phenoxybenzamine, but not different from each other (by 29.6 ± 5.0% and 37.4 ± 5.3%; $n = 5$, for noradrenaline and phenylephrine, respectively) (Figure 3c).

*Relationship between occupancy and response*

The concentration-response curves of phenylephrine used to determine the dissociation constants (before and after fractional irreversible $\alpha_1$-adrenoceptor inactivation with phenoxybenzamine) are presented in Figure 2b (for the mesenteric artery), in Figure 3c (for the renal artery) and in Figure 1d (for the saphenous vein). The $K_A$ values of phenylephrine were 4.24 (2.57–6.99) × $10^{-6}$ M ($n = 6$), 9.63 (7.06–13.1) × $10^{-6}$ M ($n = 7$), and 4.84 (3.02–7.77) × $10^{-6}$ M ($n = 6$) for the mesenteric artery, the renal artery, and the saphenous vein, respectively.

**Table 2** The occupancy-response relationships calculated for the α-adrenoceptor-mediated responses to phenylephrine

| *Saphenous vein* | | *Mesenteric artery* | | *Renal artery* | |
|---|---|---|---|---|---|
| *% response* | *% receptor occupancy* | *% response* | *% receptor occupancy* | *% response* | *% receptor occupancy* |
| 18 | 4 | 11 | 5 | 24 | 2 |
| 48 | 11 | 31 | 12 | 41 | 6 |
| 50 | 12 | 50 | 19 | 50 | 9 |
| 76 | 27 | 68 | 30 | 60 | 16 |
| 88 | 52 | 87 | 56 | 81 | 36 |
| 94 | 77 | 95 | 79 | 89 | 63 |
| | | | | 95 | 83 |

These values were obtained in the mesenteric and the renal arteries (only $\alpha_1$-adrenoceptors)) and in the saphenous vein (both $\alpha_1$- and $\alpha_2$-adrenoceptors).

The occupancy-response relationships calculated for $\alpha_1$-adrenoceptor-mediated effects of phenylephrine are shown in Table 2. It is apparent from this table that phenylephrine produces a 50% maximal response by occupying approximately 18% of available $\alpha_1$-adrenoceptors in the mesenteric artery, 9% in the renal artery, and 12% in the saphenous vein. However, for a 95% maximal response, about 80% $\alpha_1$-adrenoceptor occupancy is required in all the vessels.

## Discussion

The present study shows that in the canine saphenous vein, a tissue where postsynaptic $\alpha_1$- and $\alpha_2$-adrenoceptors coexist (De Mey & Vanhoutte, 1980; Shepperson & Langer, 1981; Polónia *et al.*, 1985), phenoxybenzamine antagonized the responses to phenylephrine more than those to noradrenaline. This differential antagonism by phenoxybenzamine may be a consequence of its preferential affinity for $\alpha_1$-adrenoceptors (Dubocovich & Langer, 1974) on the one hand, and to the relatively higher affinity of phenylephrine for $\alpha_1$- compared to $\alpha_2$-adrenoceptors, on the other hand. It is well known that noradrenaline is a mixed $\alpha_1$/$\alpha_2$-adrenoceptor agonist, while phenylephrine is a relatively selective $\alpha_1$-adrenoceptor agonist. The ratio between $EC_{50}$ values for $\alpha_2$- and $\alpha_1$-adrenoceptor-mediated responses in the rabbit pulmonary artery, for example, is 0.6 for noradrenaline and 31 for phenylephrine (Starke, 1981). This means that only at high concentrations (close to those required for maximal responses) does phenylephrine activate $\alpha_2$-adrenoceptors (Guimarães *et al.*, 1987). This may explain why phenoxybenzamine, at all concentrations used (1, 3, 10 and 30 nmol $l^{-1}$) antagonized the responses to phenylephrine more than those to noradrenaline.

Examination of the effect caused by phenoxybenzamine on the shape of the concentration-response curves to noradrenaline and phenylephrine obtained in the saphenous vein, shows that only in one situation did phenoxybenzamine cause a displacement to the right without depression of the maximum: this displacement, which occurred for the concentration-response curve of noradrenaline, was caused by 3 nmol $l^{-1}$ phenoxybenzamine and was very small (0.4 log units). For phenylephrine, such a displacement (without depression of the maximum) never occurred.

It would be tempting to conclude that a very small receptor reserve exists for noradrenaline since a parallel shift to the right occurs before a reduction in the maximum of its curve becomes manifest (Ariëns *et al.*, 1964). However, it would be entirely wrong to apply the concept of receptor reserve to the saphenous vein since two different kinds of α-adrenoceptor exist in this tissue and noradrenaline is able to activate both of them with similar efficacy. So, the parallel displacement of the concentration-response curve to the right could reveal a 'false' receptor reserve, in the sense that it can have nothing to do with a 'true' receptor reserve, which should mean the surplus of only one type of receptor.

It is not easy to explain why noradrenaline is able to induce a maximum response after 3 nmol $l^{-1}$ phenoxybenzamine, which irreversibly blocked a fraction of $\alpha_1$-adrenoceptors, yet phenylephrine, which also is able to activate both $\alpha_1$- and $\alpha_2$-adrenoceptors (Guimarães *et al.*, 1987) is not. Phenylephrine has been shown to be a partial agonist under certain conditions (Minneman & Johnson, 1984), which might explain such a difference between phenylephrine and noradrenaline, but this does not apply to the canine saphenous vein where phenylephrine has been shown to be a full agonist (Sullivan & Drew, 1980; Polónia *et al.*, 1985).

Responses to noradrenaline which are phenoxybenzamine-resistant and not mediated by typical α-adrenoceptors have been described (Laher *et al.*, 1986). However, such atypical receptors do not seem to be involved in our experiments since concentrations of phenoxybenzamine higher than 30 nmol $l^{-1}$ were not used and maximal responses to noradrenaline were obtained with concentrations below those required to activate these atypical receptors.

As a different approach for studying α-adrenoceptor reserve in vascular tissues and to avoid the role of $\alpha_2$-adrenoceptors, experiments were also carried out in the dog mesenteric and renal arteries where only $\alpha_1$-adrenoceptors exist postsynaptically, as judged by the effects of $\alpha_1$- and $\alpha_2$-adrenoceptor agonists and antagonists (Polónia *et al.*, 1985). In these two tissues, at no concentration did phenoxybenzamine shift the concentration-response curves of noradrenaline and phenylephrine to the right without depressing the maximum. Hence, when only one type of receptor is involved in the responses, i.e. mesenteric and renal arteries for both noradrenaline and phenylephrine, no functional receptor reserve was found.

Both in the renal and the mesenteric arteries, phenoxybenzamine caused a displacement of the concentration-response curves (for noradrenaline and phenylephrine) to the right as well as a depression of the maximum effect. However, while in the renal artery the reduction of the maximum effect caused by phenoxybenzamine was identical for noradrenaline and phenylephrine, in the mesenteric artery the reduction of the maximum effect of phenylephrine was distinctly larger than that of noradrenaline: 35% cf 10% (at 1 nmol $l^{-1}$ phenoxybenzamine) and 59% cf. 26% (at 3 nmol $l^{-1}$ phenoxybenzamine). Recently, Vanhoutte & Flavahan (1986a) analysed data from the literature and concluded that two subtypes of α-adrenoceptor appear to exist in the vascular smooth muscle from rodents. The differences in the reductions of the

maximum effects of phenylephrine and noradrenaline caused by phenoxybenzamine in the mesenteric and renal arteries could also be discussed on that basis. Some other data obtained in our experiments could be explained in the same light. For example: if more than one type of $\alpha_1$-adrenoceptor also existed in the vascular smooth muscle of the dog this might explain why the slope of the concentration-response curve for phenylephrine is steeper in the mesenteric than in the renal artery, as well as why the dissociation constant for phenylephrine is twice as high in the mesenteric than in the renal artery.

More recently, half-maximal responses to selective $\alpha_1$-adrenoceptor agonists were obtained in canine saphenous vein by occupying only a small fraction of the available $\alpha_1$-adrenoceptor pool (4–5%: Ruffolo & Zeid, 1985; 1%: Vanhoutte & Flavahan, 1986b) and the maximal contractile response to the same agonists could be obtained at an $\alpha_1$-adrenoceptor occupancy of 60% (Ruffolo & Zeid, 1985) or 15% (Vanhoutte & Flavahan, 1986b). In spite of the unsuitability of the canine saphenous vein for studying the $\alpha_1$-adrenoceptor reserve (since it has $\alpha_1$- and $\alpha_2$-adrenoceptors), we also determined the relationship between receptor occupancy and responses in this tissue, just for the sake of comparison. In our experiments the half-maximal response occurred at 12% $\alpha$-adrenoceptor occupancy. Any differences between the values obtained by different groups may depend on the different agonist used and/or the region of the saphenous vein removed.

All these results, which indicate that a large receptor reserve exists in canine saphenous vein, were not confirmed by the functional approach of our study, since no displacement of the concentration-response curves of phenylephrine to the right were caused by phenoxybenzamine without a reduction of the maximum effect.

In the calculations of the adrenoceptor occupancy-response relationship the authors mentioned above underestimated the presence of postsynaptic $\alpha_2$-adrenoceptors. This underestimation may introduce an important cause of error since it has recently been shown that after phenoxybenzamine, in a concentration which depresses the maximum effect of phenylephrine by about 30%, the contractions caused by selective $\alpha_1$-adrenoceptor agonists (phenylephrine and methoxamine) are predominantly mediated by $\alpha_2$-adrenoceptors (Guimarães *et al.*, 1987). This is why phenoxybenzamine cannot be used to determine the affinity of a selective $\alpha_1$-adrenoceptor agonist for $\alpha_1$-adrenoceptors when both $\alpha_1$- and $\alpha_2$-adrenoceptors exist in the same preparation, i.e. rightward displacement by phenoxybenzamine results in the loss of receptor subtype selectivity for the agonist.

To calculate the relationships between adrenoceptor-occupancy and response, in the present study, two vascular tissues were used which possess only postsynaptic $\alpha_1$-adrenoceptors: the mesenteric and the renal arteries of the dog. The results obtained show, for the smaller responses, a pronounced 'excess' of adrenoceptors, since occupation of only 9% (in the renal) and 18% (in the mesenteric) of them is required for a half-maximal response. However, what we should like to emphasize is that from a 50% maximal response onwards, the percentage of total $\alpha$-adrenoceptor pool which has to be activated for eliciting a response increases more quickly than the magnitude of the response, such that for a 95% maximal response about 80% $\alpha_1$-adrenoceptor occupancy is required. Thus the surplus $\alpha_1$-adrenoceptors which is very large for a 50% maximal response becomes smaller and smaller as the magnitude of the response approaches the 100% level.

If we retain the original definition of 'spare receptors', i.e. receptors in excess of those required to produce a maximal response (Clark, 1933; Stephenson, 1956), we must conclude that there is no receptor reserve in the mesenteric and renal arteries.

The present results support the hypothesis that the differential antagonism by calcium entry blockers of the responses mediated by vascular $\alpha_1$- and $\alpha_2$-adrenoceptors is not due to differences in receptor reserve between the postsynaptic vascular $\alpha$-adrenoceptor subtypes since, in all probability, no $\alpha_1$-adrenoceptor reserve exists in the vascular tissue, at least *in vitro*.

As shown by Guimarães *et al.* (1987), after phenoxybenzamine, which selectively inhibits $\alpha_1$-adrenoceptors, the selective $\alpha_1$-adrenoceptor agonists cease to be $\alpha_1$-selective and activate $\alpha_2$-adrenoceptors. This explains why after phenoxybenzamine the responses to $\alpha_1$-agonists become very sensitive to antagonism by calcium entry blockers.

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## References

ARIENS, E.J., SIMONIS, A.M. & VAN ROSSUM, J.M. (1964). The relation between stimulus and effect. In *Molecular Pharmacology* (Vol. 3) ed. Ariens, E.J. pp. 394–492. New York: Academic Press.

BLACK, J.W., DUNCAN, W.A.M. & SHANKS, R.G. (1965). Comparison of some properties of pronethalol and propranolol. *Br. J. Pharmac.*, **25**, 577–591.

CLARK, A.J. (1933). The Mode of Action of Drugs on Cells. London: Edward Arnold & Co.

DE MEY, J.C. & VANHOUTTE, P.M. (1980). Differences of

pharmacological properties of post-junctional alpha-adrenergic receptors among arteries and veins. *Archs. int. Pharmacodyn. Ther.*, **244**, 328–329.

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.*, **327**, 505–519.

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

FURCHGOTT, R.F. & BURSZTYN, P. (1967). Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors. *A. New York Acad. Sci.*, **144**, 882–889.

GUIMARÃES, S. (1975). Further study of the adrenoceptors of the saphenous vein of the dog: influence of factors which interfere with the concentrations of agonists at the receptor level. *Eur. J. Pharmac.*, **34**, 9–19.

GUIMARÃES, S., BRANDÃO, F. & PAIVA, M.Q. (1978). A study of the adrenoceptor-mediated feedback mechanisms by using adrenaline as a false transmitter. *Naunyn-Schmiedebergs Arch. Pharmac.*, **305**, 185–188.

GUIMARÃES, S. & OSSWALD, W. (1969). Adrenergic receptors in the veins of the dog. *Eur. J. Pharmac.*, **5**, 133–140.

GUIMARÃES, S. & PAIVA, M.Q. (1977). The role played by the extraneuronal system in the disposition of noradrenaline and adrenaline in vessels. *Naunyn-Schmiedebergs Arch. Pharmac.*, **296**, 279–287.

GUIMARÃES, S., PAIVA, M.Q. & MOURA, D. (1987). Alpha$_2$-adrenoceptor-mediated responses to so-called selective alpha$_1$-adrenoceptor agonists after partial blockade of alpha$_1$-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmac.*, **335**, 397–402.

HAMILTON, C.A., REID, J.L. & SUMNER, D.J. (1983). Acute effects of phenoxybenzamine on α-adrenoceptor responses *in vivo* and *in vitro*: Relation on *in vivo* pressor responses to the number of specific adrenoceptor binding sites. *J. cardiovasc. Pharmac.*, **5**, 863–873.

IVERSEN, L.L. & SALT, P.J. (1970). Inhibition of catecholamine uptake$_2$ by steroids in the isolated rat heart. *Br. J. Pharmac.* **40**, 528–530.

KENAKIN, T. (1986). Receptor reserve as a tissue misnomer. *Trends Pharmac. Sci.*, **7**, 93–95.

LAHER, I., NISHIMURA, S. & BEVAN, J.A. (1986). Prazosin selectively antagonizes norepinephrine contractions at low-affinity, non-alpha adrenoceptor sites (extraceptors) in arterial muscle. *J. Pharmac. exp. Ther.*, **239**, 846–852.

MINNEMAN, K.P. & JOHNSON, R.D. (1984). Characterization of alpha$_1$-adrenergic receptors linked to $^3$H-inositol metabolism in rat cerebral cortex. *J. Pharmac. exp. Ther.*, **230**, 317–323.

POLONIA, J.J., PAIVA, M.Q. & GUIMARÃES, S. (1985). Pharmacological characterization of postsynaptic α-adrenoceptor subtypes in five different dog arteries in vitro. *J. Pharm. Pharmac.*, **37**, 205–208.

RUFFOLO, R.R., MORGAN, E.L. & MESSICK, K. (1984). Possible relationship between receptor reserve and the differential antagonism of alpha$_1$ and alpha$_2$ adrenoceptor mediated pressor responses by calcium channel antagonists in the pithed rat. *J. Pharmac. exp. Ther.*, **230**, 587–594.

RUFFOLO, R.R. & YADEN, E. (1984). The existence of spare adrenoceptors for respective vasopressor effects of cirazoline and B-HT 933 in the pithed rat. *J. cardiovasc. Pharmac.*, **6**, 1011–1019.

RUFFOLO, R.R. & ZEID, R.L. (1985). Relationship between alpha adrenoceptor occupancy and response for the alpha-1 adrenoceptor agonist, cirazoline and the alpha-2 adrenoceptor agonist, B-HT 933, in canine saphenous vein. *J. Pharmac. exp. Ther.*, **235**, 636–643.

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

STARKE, K., ENDO, T. & TAUBE, M. (1975). Relative pre- and postsynaptic potencies of α-adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn-Schmiedebergs Arch. Pharmac.*, **29**, 55–78.

SASTRE, A. & MILNOR, W.R. (1985). Spare receptors: a concept revisited. In *Trends in Autonomic Pharmacology*. ed. Kalsner, S. pp. 61–70. London: Taylor and Francis.

SHEPPERSON, N.B. & LANGER, S.Z. (1981). The effects of the z-amino-tetrahydronaphthalene derivative M7, a selective $\alpha_2$-adrenoceptor agonist in vitro. *Naunyn-Schmiedebergs Arch. Pharmac.*, **318**, 10–13.

STEPHENSON, R.P. (1956). Modification of receptor theory. *Br. J. Pharmac. Chemother.*, **11**, 379–393.

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

TRENDELENBURG, U. (1966). Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **18**, 629–640.

VANHOUTTE, P.M. & FLAVAHAN, N.A. (1986a). $\alpha_1$-Adrenoceptors subclassification in vascular smooth muscle. *Trends Pharmac. Sci.*, **7**, 347–349.

VANHOUTTE, P.M. & FLAVAHAN, N.A. (1986b). Effects of temperature on α-adrenoceptors in limb veins: role of receptor reserve. *Fedn. Proc.*, **45**, 2347–2354.

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