J. Pharm. Pharmacol. 1986, 38: 600–605
Received November 11, 1985

Pharmacological characterization of the postsynaptic α-adrenoceptors in human uterine artery*

C. A. FONTES RIBEIRO AND TICE A. MACEDO†

Instituto de Farmacologia e Terapêutica Experimental, Faculdade de Medicina, Universidade de Coimbra, 3000 Coimbra, Portugal

Prazosin and yohimbine were used to differentiate postjunctional α-adrenoceptors in the human uterine artery in-vitro. Two postjunctional α-adrenoceptor subtypes were distinguished by the affinities of the receptor for yohimbine and prazosin. The pA_2 for prazosin was 8·91 against phenylephrine with a slope not significantly different from unity (0·91), and the pA_2 for yohimbine was 7·25 against naphazoline and 8·70 against clonidine, with slopes not significantly different from unity (1·11 and 1·18, respectively). Yohimbine was not very active against phenylephrine, while prazosin was very active against the mixed and selective α_2-adrenoceptor agonist noradrenaline and clonidine; the intercepts of the Schild plot were 8·80 and 8·82 but with slopes significantly less than unity (0·77 and 0·67, respectively). Prazosin competitively antagonized phenylephrine at the α_1-adrenoceptor, whereas yohimbine competitively antagonized naphazoline and clonidine at the α_2-adrenoceptor. It is concluded that both α_1- and α_2-adrenoceptors are present in the human uterine artery.

Catecholamines exert their contractile responses through α-adrenoceptor stimulation (Docherty et al 1979). The characterization of α-adrenoceptors may be carried out by pharmacological means, using relatively selective agonists and antagonists, and by direct measurements of radioligand binding to receptors in subcellular fractions. According to recent results, postsynaptic α-adrenoceptors of the vessels can be divided into two subgroups: α_1- and α_2-adrenoceptors (De Mey & Vanhoutte 1981; Langer & Shepperson 1982; Shoji et al 1983).

α_1-Adrenoceptors are found in most, if not all, blood vessels but there is a marked diversity in the distribution of postsynaptic α_2-adrenoceptors among blood vessels from several species (Wikberg 1979; Timmermans & van Zwieten 1981; Langer & Shepperson 1982; Marwaha & Aghajanian 1982; McGrath 1982, 1983; Shoji et al 1983; Polónia et al 1985). Besides, although much evidence has been obtained from in-vivo studies, evidence from in-vitro arterial preparations to support the existence of these two postsynaptic α-adrenoceptors is less impressive (McGrath 1982, 1983; Moore & Griffiths 1982; Digges & Summers 1983; Shoji et al 1983; Medgett & Langer 1984; Polónia et al 1985). This difficulty may arise from the variability in the ratio of postsynaptic α_1- and α_2-adrenoceptors between different vascular beds and from the type of blood vessels (large capacitance versus small resistance blood vessels) (Langer & Shepperson 1982; Digges & Summers 1983). Some studies (De Mey & Vanhoutte 1981; Shepperson & Langer 1981; Fowler et al 1984; Docherty & Hyland 1985) have demonstrated that the saphenous vein is an exception to this difficulty. But other vessels or tissues may also be exceptions.

In the present paper we report the results obtained in isolated strips of human uterine arteries.

MATERIALS AND METHODS

Human uterine arteries were obtained from patients undergoing surgery for myomatosis. All the women were otherwise healthy, normotensive and without drug treatment. The hysterectomies were carried out during the follicular phase of the cycle under thiopentone-halothane anaesthesia. Segments of macroscopically normal arteries, 1–2 mm in outer diameter in-situ, were dissected free and removed and care was taken to avoid stretching or other type of injury. All vessel segments were immediately placed in chilled Krebs–Henseleit solution (composition in mM: NaCl 118·67; KCl 5·36, $MgSO_4.7H_2O$ 0·57, $CaCl_2$ 1·90, $NaH_2PO_4.2H_2O$ 0·90, $NaHCO_3$ 25·0, glucose 11·1, ascorbic acid 0·06 and Na_2 EDTA 0·03). Excess fat and connective tissue were trimmed off and the artery was cut into helical strips measuring about 25 mm in length. Then the vascular segments were suspended in 50 ml tissue baths which were maintained at 37·0 °C, pH 7·4, and aerated

* Preliminary data were presented at the 5th Meeting on Adrenergic Mechanisms, October 1983, in Porto, Portugal.
† Correspondence.

continuously with a 5% CO_2–95% O_2 mixture. The tissues were allowed to equilibrate for 2 h under a basal tension of 2 g before experimentation. The bath solution was replaced several times during the equilibration period. Isotonic contractions were recorded on smoked paper with a magnification of approximately 10 times. Cocaine (10 μM), to block neuronal uptake mechanisms, propranolol (2·8 μM), to block β-adrenoceptors, and cortexone (10 μM), to inhibit extraneuronal uptake, were present in the organ bath solution (Fowler et al 1984; Agrawal et al 1984; Steen et al 1984).

To begin the experiment a maximal response to noradrenaline was determined in order to verify the reactivity of the tissue and to compare with noradrenaline the contractions obtained with the other agonists. Then two cumulative dose-response curves were performed per preparation and whenever each response attained the maximum, the agonist was added such that the final concentration in the bath each time was increased by a factor of 3. The second dose-response curve was performed after a 45 min exposure to prazosin or yohimbine. In the same day at least three different concentrations of the antagonist were used but with only one concentration of antagonist per strip. In all experiments one tissue strip was run in parallel but without any antagonist to check any time-dependent changes in sensitivity to the agonist.

Experiments with clonidine and naphazoline were conducted under a sodium lamp.

Analysis of data

All concentration-response (cr) curves were plotted graphically and E_{max}, i.e. the maximum contraction obtained with an agonist, and EC50, i.e. the agonist concentration at which 50% of maximum effect occurs, were calculated. pD_2 is defined as the negative logarithm of the EC50 value. pA_2, i.e. the negative logarithm of the antagonist concentration that displaces the agonist cr-curves towards higher concentrations with a factor of 2, was determined according to the method of Arunlakshana & Schild (1959). The concentration ratio (CR) was calculated as the ratio between the EC50-values in the presence and absence of antagonist in the same strip. For prazosin against naphazoline, pA_2 was also calculated according to the method described by Furchgott (1972), i.e. pA_2 equals the negative logarithm of the dissociation constant K_B of the receptor-antagonist complex:

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

Statistics

Results are expressed as the arithmetic mean ± s.e.m. Significances of difference were performed using Student's *t*-test ($P < 0.05$) or by testing for overlap of 95% confidence limits. All straight lines were drawn by linear regression.

Drugs used

(±)-Noradrenaline; naphazoline HCl; (−)-phenylephrine HCl; yohimbine HCl; (±)-propranolol HCl and cortexone (desoxycorticosterone) were obtained from Sigma Chemical Co (St Louis, USA). Prazosin HCl was a gift from Laboratorio Pfizer (Lisbon, Portugal) and clonidine HCl was supplied by C. H. Boehringer Sohn L.da (Lisbon, Portugal). Cocaine HCl was obtained from E. Merck (Darmstadt, West Germany).

All the agonists were dissolved in 0·01 M HCl and prepared daily. Yohimbine was dissolved in distilled water after warming. Prazosin was dissolved in 50% propandiol and diluted with Krebs-Henseleit solution. Cortexone was also dissolved in 50% propandiol/distilled water.

RESULTS

There was tachyphylaxis when concentration-response curves (cr-curves) were repeated for phenylephrine and clonidine. This fact was taken into account to correct the cr-curves determined in the presence of antagonists. For noradrenaline and naphazoline there was no tachyphylaxis.

Effects of α-adrenoceptor agonists.

The pD_2 and E_{max} values are given in Table 1. The maximal responses elicited by clonidine were smaller (51 ± 4% of noradrenaline contractions) than those caused by the other agonists which were equieffective in our preparation. The time to reach steady state for each concentration was greater for naphazoline and clonidine than for noradrenaline

Table 1. Mean values ± s.e.m. of the negative logarithm of the ED50 (pD_2) and of the maximum contraction obtained with the agonist in the first cumulative concentration-response curve in comparison with the maximal stimulation obtained with the ED100 of noradrenaline (=1) in the beginning of the experiment. Cocaine (10 μM), cortexone (10 μM) and propranolol (2·8 μM) were present in the bath.

Agonist	pD_2	E_{max}
Noradrenaline	5·45 ± 0·20	0·99 ± 0·02
Phenylephrine	4·80 ± 0·27	0·98 ± 0·05
Naphazoline	6·40 ± 0·25	0·96 ± 0·10
Clonidine	6·00 ± 0·30	0·52 ± 0·04

and phenylephrine whereas the noradrenaline and phenylephrine cr-curves were steeper. The pEC50 or pD_2 values, calculated by normalizing the data on each agonist, indicate that the rank order of potency was naphazoline > clonidine > noradrenaline > phenylephrine (Table 1), which suggest the presence of an α_2-adrenoceptor subtype.

Effects of α-adrenoceptor antagonists.

Prazosin, an α_1-selective antagonist, markedly shifted to the right the noradrenaline, phenylephrine, naphazoline and clonidine cr-curves (Figs 1, 2, 3, 4); the calculated pA_2 or X-axis intercept are shown in

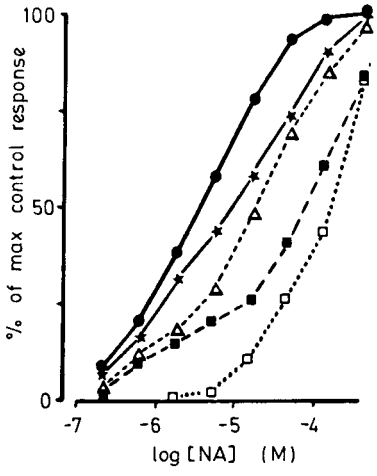

FIG. 1. Concentration-contractile response curve obtained for noradrenaline (NA) in the absence and presence of various concentrations of prazosin added 45 min before addition of the agonist. Cocaine, propranolol and cortexone are always present in the bath (see methods). Each point is the mean of five to twelve experiments. Standard errors are excluded for clarity and do not exceed 10% of the mean value for each point. Key: (●) control, (★) 0·003 μM, (△) 0·01 μM, (■) 0·10 μM, (□) 0·40 μM prazosin.

Table 2. The slope of the Schild plot obtained against phenylephrine (Table 2) did not differ significantly from unity, which is consistent with a competitive antagonism. The slopes obtained for prazosin against noradrenaline and clonidine (Table 2), significantly differed from unity; taken together with the pA_2 this may indicate that these agonists are stimulating more than one adrenoceptor subtype. However, prazosin had little effect on the lower portion of the cr-curve of clonidine, in contrast to yohimbine.

Yohimbine, a reported α_2-selective antagonist, exhibited an antagonism of noradrenaline-, naphazoline- and clonidine-induced contractions (Figs 5, 6, 7) with little effect on phenylephrine-

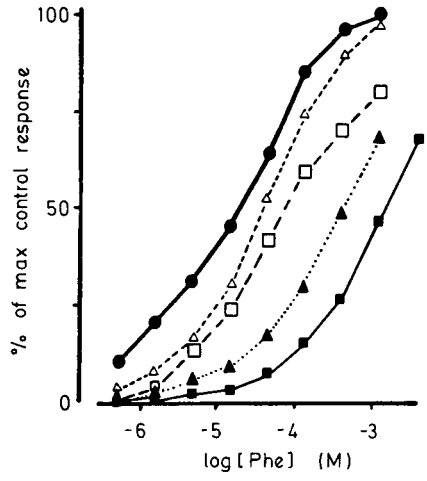

FIG. 2. Cumulative concentration-response curves for phenylephrine (Phe) in the absence (control) and presence of various concentrations of prazosin, added 45 min before the performance of the second concentration-response curve to phenylephrine. Cocaine, propranolol and cortexone are always present (see methods). Each point represents the mean of five to twelve experiments. Standard errors are excluded for clarity and do not exceed 15% of the mean value for each point. Key: (●) control, (△) 0·001 μM, (□) 0·01 μM, (▲) 0·03 μM, (■) 0·10 μM prazosin.

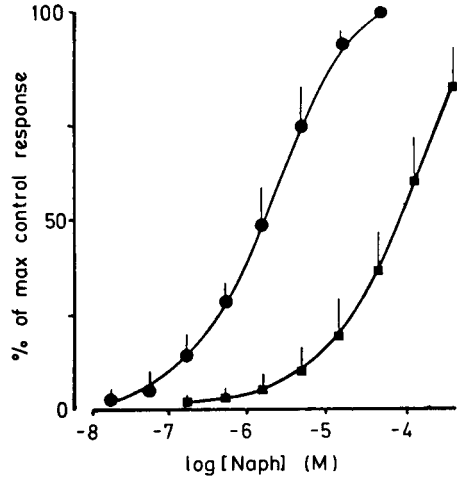

FIG. 3. Effect of prazosin on naphazoline (Naph)-induced contractions. Prazosin was added to the bath 45 min before performing the second cumulative concentration-response curve. Each point and bar represents mean ± s.e.m. of four to six experiments. Key: (●) control; (■) 4 μM prazosin.

elicited responses (Fig. 8). The calculated pA_2 values or X-axis intercepts and slopes of the Schild plots for noradrenaline, phenylephrine, naphazoline and clonidine are shown in Table 2.

These results indicate that noradrenaline stimulates more than one subtype of adrenoceptor in the

Table 2. $pA_2 \pm$ s.e.m. values obtained in the present study for the interaction of prazosin and yohimbine with various α-adrenoceptor agonists in isolated smooth muscle segments of the human uterine artery.

	Agonist	pA_2 Value	Slope of regression line
Prazosin	Noradrenaline	8·80 ± 0·15[a]	0·77 ± 0·05[b]
	Phenylephrine	8·91 ± 0·12	0·91 ± 0·09
	Naphazoline	7·05 ± 0·10[c]	
	Clonidine	8·82 ± 0·27[a]	0·67 ± 0·15[b]
Yohimbine	Noradrenaline	7·27 ± 0·11[a]	0·69 ± 0·08[b]
	Phenylephrine	6·17 ± 0·23[a]	0·48 ± 0·12[b]
	Naphazoline	7·25 ± 0·14	1·11 ± 0·09
	Clonidine	8·70 ± 0·35	1·18 ± 0·19

[a] As the slope of the Schild plot is significantly different from unity, these values should not strictly be regarded as pA_2 but rather should be called intercepts of the Schild plot.
[b] The slope is significantly different from unity, $P < 0.05$.
[c] K_B (method of Furchgott 1972).

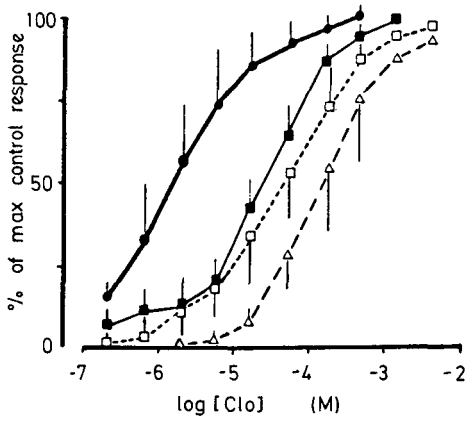

FIG. 4. Effects of prazosin on clonidine(Clo)-induced contractions. Prazosin was added to the bath 45 min before performing the second concentration-response curve. Each point represents the mean ± s.e.m. of six to ten tissues. Experiments were performed in the dark (sodium lamp). Key: (●) control, (■) 0·10 μM, (□) 0·40 μM, (△) 1·00 μM prazosin.

human uterine artery, one of these subtypes being preferentially stimulated by naphazoline and clonidine, since yohimbine had a weak activity against phenylephrine but competitively antagonized the naphazoline- and clonidine-induced contractions (slopes not significantly different from unity).

DISCUSSION

In the present report, in isolated strips of human uterine arteries, the postjunctional α-adrenoceptors have been characterized on the basis of their sensitivity to phenylephrine, a selective α_1-adrenoceptor agonist (Cambridge et al 1977; Berthelsen & Pettinger 1977; Starke 1981; Moore & Griffiths 1982; Langer & Shepperson 1982), naphazoline and clonidine, selective α_2-adrenoceptor agonists (Starke et al 1975b; Berthelsen & Pettinger 1977; Starke & Docherty 1980; Langer & Shepperson 1982; Vanhoutte 1982), and noradrenaline, a

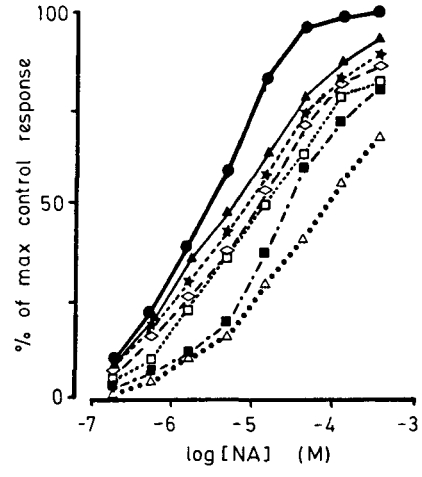

FIG. 5. Effect of yohimbine on noradrenaline (NA)-induced contractions. Yohimbine was present in the bath 45 min before addition of the agonist. Cortexone, propranolol and cocaine were always present (see methods). Each point represents the mean of five to twelve tissues. Standard errors are excluded for clarity and do not exceed 10% of the mean value for each point. Key: (●) control; (▲) 0·05 μM, (★) 0·14 μM, (◇) 0·40 μM, (□) 0·50 μM, (■) 2·00 μM, (△) 5·00 μM yohimbine.

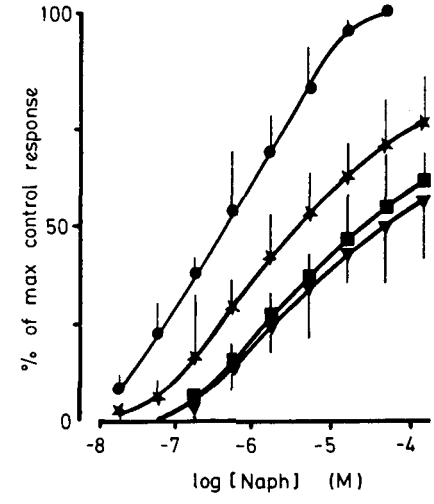

FIG. 6. Effect of yohimbine on naphazoline(Naph)-induced contractions. Yohimbine was present in the bath 45 min before naphazoline. Each point represents the mean ± s.e.m. of five tissues. Key: (●) control, (★) 0·4 μM, (■) 2·0 μM, (▼) 4·0 μM yohimbine.

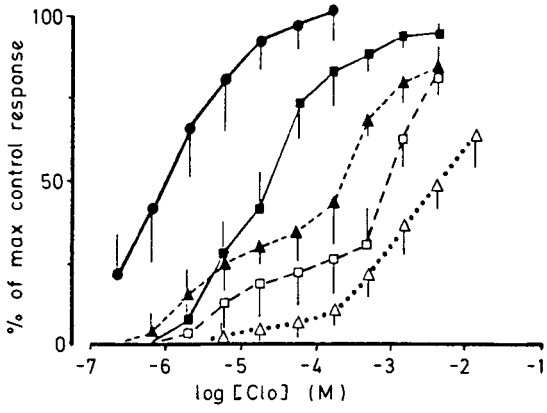

FIG. 7. Effect of yohimbine on clonidine (Clo)-induced contractions. Yohimbine was added to the bath 45 min before performing the second cumulative concentration-response curve of clonidine. Each point represents the mean ± s.e.m. of six to ten tissues. Experiments were performed under a sodium lamp. Key: (●) control; (■) 0·05 μM; (▲) 0·20 μM; (□) 0·50 μM; (△) 5·00 μM yohimbine.

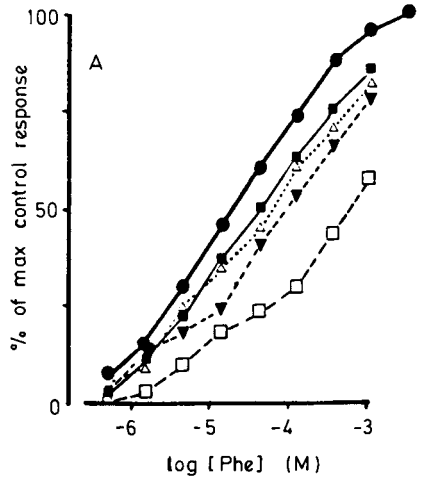

FIG. 8. Cumulative concentration-response curves to phenylephrine (Phe) in the absence (control) and presence of yohimbine at various concentrations, added 45 min before addition of the agonist. Cocaine, propranolol and cortexone are always present (see methods). Each point represents the mean of five to twelve tissues. Standard errors are excluded for clarity but do not exceed 15% of the mean value of each point. Key: (●) control, (■) 0·5 μM, (△) 5·0 μM, (▼) 50·0 μM, (□) 250·0 μM yohimbine.

mixed agonist (Fowler et al 1984), as well as to prazosin, an α_1-adrenoceptor antagonist (Cambridge et al 1977; Williams et al 1981; Langer & Shepperson 1982; Steen et al 1984) and yohimbine, an α_2-adrenoceptor antagonist (Starke et al 1975a; Wood et al 1979; Langer & Shepperson 1982).

Our results show that noradrenaline, phenylephrine and naphazoline were full agonists, whereas the maximal contraction caused by clonidine was about 50% of the E_{max} of noradrenaline. Different data (Digges & Summers 1983; Agrawal et al 1984; Decker & Schwartz 1985) have shown clonidine as a partial agonist, which can explain this effect.

Prazosin shifted to the right the concentration-response curve of all agonists. The pA_2 or X-axis intercept obtained are close to those reported for the existence of α_1-adrenoceptors. They range between approximately 8·0 and 9·0 (Agrawal et al 1984). These results may allow us to conclude that naphazoline and clonidine were not selective enough for α_2-adrenoceptors, or the human uterine artery has no α_2-adrenoceptors. However, yohimbine had little effect against phenylephrine, while it shifted to the right the cr-curves of noradrenaline, naphazoline and clonidine. If there were no α_2-adrenoceptors, yohimbine should have been equally potent against the different agonists. Thus, the present results suggest that the human uterine artery contains two populations of postsynaptic α-adrenoceptors, and that clonidine and naphazoline are not specific for α_2-adrenoceptors; this agrees with the views of Decker et al (1984) but disagrees with those of van Meel et al (1981) who used a different experimental model. In conformity with this conclusion the slopes of the Schild plots obtained for prazosin against noradrenaline and clonidine were significantly less than unity (for naphazoline we used only one concentration of prazosin), which suggests the presence of more than one subtype of receptor activated by those agonists (Kenakin 1985). For phenylephrine, the slope of the Schild plot was close to unity, indicating that agonist and antagonist may interact competitively at the same receptor (Kenakin 1985), i.e. an α_1-adrenoceptor.

Yohimbine shifted to the right the cr-curves of noradrenaline, naphazoline and clonidine. The pA_2 or X-axis intercept obtained are similar to those reported as indicating the existence of α_2-adrenoceptors. The slopes of the Schild plot were significantly different from unity when noradrenaline and phenylephrine were used, whereas such slopes are near unity when naphazoline and clonidine were employed; once more this indicates that antagonist and agonists may interact competitively at the same receptor (Kenakin 1985), i.e. an α_2-adrenoceptor. These facts demonstrate the existence of a mixture of two receptor subtypes (α_1 and α_2), with the preponderance of one of them (Kenakin 1985), the α_1-adrenoceptor subtype.

In conclusion, the human uterine artery in-vitro appears to be the second blood vessel with demonstrable α_2-adrenoceptors.

We have used tissues from women in the oestrogen phase of the cycle and Hoffman et al (1981) and Larsson et al (1984) have demonstrated that oestrogen increases the number of postjunctional α_2-adrenoceptors. It will be interesting to verify whether the human uterine artery in a non-oestrogen phase has the same characteristics.

Acknowledgements

We would like to thank the gynaecologists and nurses of Instituto Materno-Infantil Doutor Bissaya Barreto and the Hospitais da Universidade de Coimbra for their cooperation in providing the specimens.

Thanks are given to J. M. C. Vinhas, F. Rovira and C. Santos for technical assistance. The research was supported by INIC 83/CM/12.

REFERENCES

Agrawal, D. K., Triggle, C. R., Daniel, E. E. (1984) J. Pharmacol. Exp. Ther. 229: 831–838

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. 14: 48–59

Berthelsen, S., Pettinger, W. A. (1977) Life Sci. 21: 595–606

Cambridge, D., Davey, M. J., Massingham, R. (1977) Br. J. Pharmacol. 59: 514P–515P

De Mey, J., Vanhoutte, P. M. (1981) Circ. Res. 42: 875–884

Decker, N., Ehrhardt, J. D., Leclerc, G., Schwartz, J. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 326: 1–6

Decker, N., Schwartz, J. (1985) J. Pharmacol. Exp. Ther. 222: 251–257

Digges, K. G., Summers, R. J. (1983) Br. J. Pharmacol. 79: 655–665

Docherty, J. R., MacDonald, A., McGrath, J. C. (1979). Ibid. 67: 421P–422P

Docherty, J. R., Hyland, L. (1985) Ibid. 84: 573–576

Fowler, P. J., Grous, M., Price, W., Matthews, W. D. (1984) J. Pharmacol. Exp. Ther. 229: 712–718

Furchgott, R. R. (1972) in: Blaschko, H., Muscholl, E. (eds) Catecholamines, Handbook of Experimental Pharmacology, vol. 33. Springer Verlag, New York pp 283–355

Hoffman, B. B., Lavin, T. N., Lefkowitz, R. J., Ruffolo, R. R., Jr. (1981) J. Pharmacol. Exp. Ther. 219: 290–295

Kenakin, T. P. (1985) Trends Pharmacol. Sci. 6: 68–71

Langer, S. Z., Shepperson, N. B. (1982) Ibid. 3: 440–444

Larsson, B., Anderson, K.-E., Batra, S., Mattiasson, A., Sjogren, C. (1984) J. Pharmacol. Exp. Ther. 229: 557–563

Marwaha, J., Aghajanian, G. K. (1982) Ibid. 222: 287–293

McGrath, J. C. (1982) Biochem. Pharmacol. 31: 467–484

McGrath, J. C. (1983) Trends Pharmacol. Sci. 4: 14–18

Medgett, I. C., Langer, S. Z. (1984) J. Pharmacol. Exp. Ther. 229: 823–830

Moore, P. K., Griffiths, A. J. (1982) Arch. Int. Pharmacodyn. 260: 70–77

Polónia, J. J., Paiva, M. Q., Guimarães, S. (1985) J. Pharm. Pharmacol. 37: 205–208

Shepperson, N. B., Langer, S. Z. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 318: 10–13

Shoji, T., Tsuru, H., Shigei, T. (1983) Ibid. 324:248–253

Starke, K. (1981) Rev. Physiol. Biochem. Pharmacol. 88: 199–236

Starke, K., Docherty, J. R. (1980) J. Cardiovasc. Pharmacol. 2 (suppl.): 5269–5286

Starke, K., Borowski, E., Takahiko, E. (1975a) Eur. J. Pharmacol. 34: 385–388

Starke, K., Endo, T., Taube, H. D. (1975b) Naunyn-Schmiedeberg's Arch. Pharmacol. 291: 55–78

Steen, S., Sjoberg, T., Skarby, T. V. C., Norgren, L., Andersson, K.-E. (1984) Acta Physiol. Scand. 122: 323–329

Timmermans, P. B. M. W. M., van Zwieten, P. A. (1981) J. Auton. Pharmacol. 1: 171–183

van Meel, J. C. A., de Jonge, A., Timmermans, P. B. M. W. M., van Zwieten, P. A. (1981) J. Pharmacol. Exp. Ther. 219: 760–767

Vanhoutte, P. M. (1982) J. Cardiovasc. Pharmacol. 4 (suppl.) 1: 591–596

Wikberg, J. E. P. (1979) Acta Physiol. Scand. 468 (Suppl.): 11–99

Williams, R. S., Dukes, D. F., Lefkowitz, R. J. (1981) J. Cardiovasc. Pharmacol. 3: 522–531

Wood, C. L., Arnett, C. D., Clarke, W. R., Tsai, B. S., Lefkowitz, R. J. (1979) Biochem. Pharmacol. 28: 1277–1282