co-administered ^{99m}Tc tablet, which is located in the stomach at t=21 min. The ^{99m}Tc marker can also be seen in this image.

The radiation dose to the stomach wall from one administration of 37 MBq ^{81m}Kr drink is 0·007 mGy (Medical Internal Radiation Dose Dosimetry System, Society of Nuclear Medicine, USA). Because of the low doses involved, a number of radiolabelled drinks may be given throughout a gastrointestinal transit study day to determine the position of the stomach. This will be particularly useful for multiple unit dosage forms where quantitative data for the percentage of the dosage form remaining in the stomach with time are required and hence a precise knowledge of stomach delineation and location is available throughout the study.

References

Clarke, G. M. (1989) Gastrointestinal Transit of Spherical Granules of Differing Size and Density. Ph.D. Thesis, University of London

Davenport, H. W. (1973) In: Physiology of the Digestive Tract. 3rd edn, Year Book Medical Press, Chicago, pp 45, 49

Davis, S. S., Nornig-Christensen, F., Khosla, R., Feely, L. C. (1988) Gastric emptying of large single unit dosage forms. J. Pharm. Pharmacol. 40: 205–207

Devereux, J. E. (1987) Gastrointestinal Transit of Multiple Unit Dosage Forms. Ph.D. Thesis, University of London

Feely, L. C., Davis, S. S. (1989) Correlation of phenylpropanolamine bioavailability with gastrointestinal transit by scintigraphic monitoring of ^{111}In-labelled hydroxypropyl methylcellulose matrices. Pharm. Res. 6: 274–278

Hardy, J. G., Wilson, C. G., Wood, E. (1985) Drug delivery to the proximal colon. J. Pharm. Pharmacol. 37: 874–877

Maublant, J. C., Sournac, M., Aiache, J. M., Veyre, A. (1987) Dissolution rate and transit times of 99m Tc DTPA labelled tablets. Eur. J. Nucl. Med. 15: 143–145

Rees, W. D. W., Go, V. L. W. Malageleda, J.-R. (1979) Simultaneous measurement of antroduodenal motility, gastric emptying and duodenogastric reflux in man. Gut 20: 963–970

J. Pharm. Pharmacol. 1992, 44: 129–132
Communicated May 17, 1991

Contractions induced by phenylephrine and noradrenaline are differently affected by endothelium-dependent relaxation in rat aorta

M. AUGUET, R. TRICOCHE*, P. BRAQUET, *Institut Henri Beaufour Research Labs, ZA de Courtaboeuf, 1 ave des Tropiques, 91952 Les Ulis Cedex, France, * CNRS UA 290, Faculté des Sciences, 40 avenue du Recteur Pineau, 86022 Poitiers, France*

Abstract—In rings of rat aorta precontracted with phenylephrine (10 μM) or noradrenaline (10 μM), addition of carbachol (10 μM) produced an endothelium-dependent relaxation. However, regardless of the concentration of agonist tested, both the intensity and duration of the relaxation were significantly less when noradrenaline, rather than phenylephrine, was used as the precontracting agent. The different responses observed do not appear to be related to destruction of endothelium-derived relaxing factor by autoxidation of noradrenaline since neither EDTA (30 μM) nor superoxide dismutase (30 units mL^{-1}) improved the relaxation to carbachol. In addition, in endothelium-free rings, the noradrenaline (1 μM)-induced contraction was less sensitive than the phenylephrine (1 μM)-induced contraction to sodium nitroprusside (0·1 μM) or to 8-Br-cGMP (300 μM). With phenylephrine-, but not noradrenaline-, induced contraction, the relaxation triggered by carbachol was significantly reduced by pretreatment of the aortic rings with chloroethylclonidine (50 μM), which inactivates a subpopulation of α_1-adrenoceptors. Thus, the results confirm that both alkylation sensitive and resistant α_1-adrenoceptors exist in rat aorta and indicate that EDRF may discriminate between these two α_1-adrenoceptor subtypes which are differently affected by phenylephrine and noradrenaline.

Since the original experiments described by Furchgott & Zawadzki (1980), the addition of a cholinergic agonist to preconstricted preparations enables the demonstration of a functional endothelium. Once stimulated by the muscarinic agonist, the endothelial cells release an endothelium derived relaxing factor (EDRF), identified as nitric oxide (Palmer et al 1987), which induces the relaxation of the underlying smooth muscle through activation of soluble guanylate cyclase. However, the nature of the contractile mechanism elicited by the precontracting agent may influence the vasodilatory properties of EDRF (Furchgott et al 1981). Thus, in rabbit aorta, the acetylcholine-induced relaxation was diminished when K^+, instead of noradrenaline, was used as precontracting agent. In rat aorta, endothelium removal differently affected the contractile response to various α-adrenoceptor agonists such as noradrenaline and clonidine (Egleme et al 1984; Lues & Schümann 1984; Martin et al 1986). In this artery, it was also demonstrated that among the different contractile events elicited by adrenoceptor agonists such as intracellular Ca^{2+} release and extracellular Ca^{2+} influx through receptor-dependent channels and potential-dependent channels. This latter process was less sensitive to EDRF (Auguet et al 1989a, b).

More recent studies have indicated that the α-adrenoceptor receptors of rat aorta do not constitute a homogeneous population (Han et al 1990; Oriowo & Bevan 1990; Piascik et al 1990). This has been experimentally demonstrated by use of chloroethylclonidine, an alkylating agent, which inactivates a subpopulation of α_1-adrenoceptors (Johnson & Minneman 1987). From these results it has been postulated that α-adrenoceptors of rat aorta are composed of α_{1A}-subtypes, insensitive to alkylation by chloroethylclonidine, and α_{1B}-subtypes, inactivated by chloroethylclonidine, according to the classification of Morrow & Creese (1986). The contractile processes elicited by α_{1A}-adrenoceptor activation are largely dependent on Ca^{2+} influx through potential-dependent channels (Han et al 1987, 1990).

In this present study, we investigated the effect of pretreatment with chloroethylclonidine on endothelium-dependent relaxation in rat aorta. For this purpose, two full α_1-adrenoceptors agonists (noradrenaline and phenylephrine) were used.

Materials and methods

Thoracic aortae were excised from male Sprague-Dawley rats killed by cervical dislocation (270–360 g, Charles River, Paris) and cleaned of surrounding tissue. Rings 2 mm wide were cut under a dissecting lens and were suspended in organ baths containing 10 mL of physiological solution (for composition see below) under a tension of 2 g at 37°C and gassed with 95% O_2–5% CO_2. Contractile responses were measured by using force-displacement transducers (Statham UC_2) coupled to a Gould

Correspondence: M. Auguet, Institut Henri Beaufour Research Labs, ZA de Courtaboeuf, 1 avenue des Tropiques, 91952 Les Ulis Cedex, France.

8000 S polygraph. In some experiments, the endothelium was disrupted mechanically by gently rubbing the luminal surface of the rings with small forceps. A 1 h equilibration period was allowed before experimentation. Normal physiological solution was composed of (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, glucose 11. Two matched rings taken from the same animal were used. In the first series of experiments one ring was precontracted with noradrenaline and the other with phenylephrine; carbachol (10 μM) a full EDRF relaxing agonist (Furchgott & Cherry 1984) was introduced when the agonist had exerted its maximal effect. After a washout period of 45 min, carbachol was retested on the ring precontracted with the other agonist. In the other series of experiments the preparations were subjected to phenylephrine (1 μM) and carbachol was tested in order to evaluate the presence or absence of endothelium. After a washout period of 45 min, one of the paired rings was precontracted with phenylephrine and the other with noradrenaline then sodium nitroprusside, 8-bromocyclic GMP (8-Br-cGMP) or carbachol (10 μM) was added. In some cases, EDTA or superoxide dismutase was added 5 min before the second application of agonist. In another set of experiments following the control of endothelium integrity, the rings were incubated with chloroethylclonidine (50 μM) for 30 min. Then the preparations were washed and after a 1 h rest period a contraction/relaxation protocol was established.

Drugs. 8-Bromoguanosine 3′5′-cyclic monophosphate, carbachol, ethylenediamine tetraacetic acid, phenylephrine, sodium nitroprusside, superoxide dismutase were from Sigma (France) and noradrenaline was purchased from Fluka (Switzerland). All drugs were prepared in deionized water.

Statistics. Results of experiments are expressed as mean ± s.e.m. of force (g). Comparisons were made by an analysis of variance, P values $\leq 0{\cdot}05$ were regarded as significant.

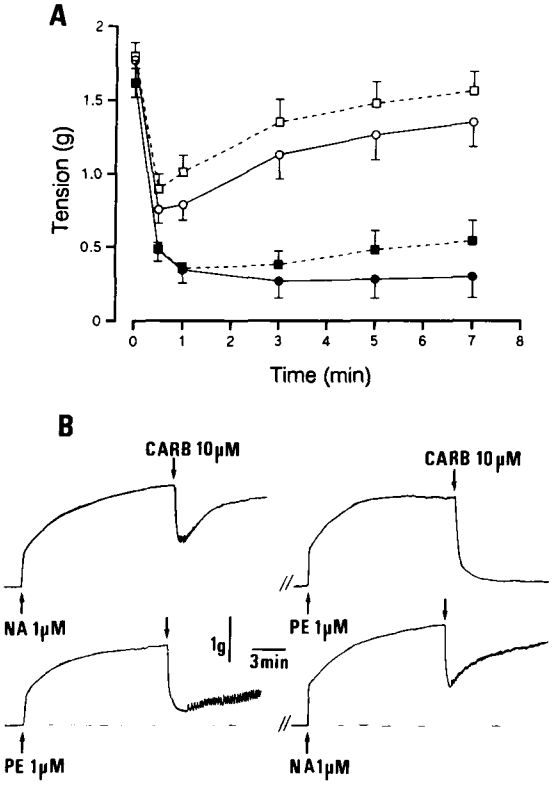

FIG. 1. A. Relaxing effect of carbachol (10 μM) added to the organ bath at t = 0 min on rat aorta precontracted with adrenaline (○, □) or phenylephrine (●, ■) at concentrations of 1 (○, ●, n = 8) or 10 μM (□, ■, n = 8). Mean ± s.e.m. (n = number of experiments.) B. Representative recordings of the method used to study the relaxing effect of carbachol on phenylephrine- and noradrenaline-precontracted aorta. The two rings are from the same rat.

Results

In aortic rings with an intact endothelium, phenylephrine (1 μM; 1·6 ± 0·09 g, n = 8) and noradrenaline (1 μM; 1·8 ± 0·13 g, n = 8) elicited similar tone. In the precontracted preparations carbachol (10 μM) induced relaxation as illustrated by the original recordings shown in Fig. 1. However, this relaxation was significantly reduced both in magnitude and duration when noradrenaline rather than phenylephrine, was used as the precontracting agent. The difference between the effect of phenylephrine and noradrenaline remained significant even if the concentration of phenylephrine used was 10-fold that of noradrenaline ($P < 0{\cdot}05$ and $P < 0{\cdot}01$ for comparison of tensions between phenylephrine, 10 μM, and noradrenaline, 1 μM, at 0·5 and 7 min, respectively).

The relaxation elicited by carbachol was not modified by EDTA (30 μM) or superoxide dismutase (30 units mL^{-1}), which retard the oxidation of catecholamines and prevent the destruction of EDRF, respectively (Fig. 2). In addition, sodium nitroprusside and 8-Br-cGMP induced a significantly smaller relaxation following contraction induced by noradrenaline (1 μM) in comparison with that elicited by phenylephrine (1 μM) (Fig. 3). As to EDRF, these two endothelium-independent relaxing agents induce relaxation through cGMP-dependent protein phosphorylation (Rapoport et al 1983).

Following treatment with 50 μM chloroethylclonidine, higher concentrations of agonists (1 mM for noradrenaline and 3 mM for phenylephrine) were required to obtain a tone similar to control preparations stimulated with 1 μM of agonist. Under these conditions, the vasorelaxant response to carbachol was unaffected in noradrenaline-preconstricted rings but was significantly reduced in phenylephrine-preconstricted tissues ($P < 0{\cdot}01$ and $P < 0{\cdot}05$ for comparison of tensions between phenylephrine-contracted preparations treated or not with chloroethylclonidine at 0·5 and 3 min, respectively) (Fig. 4).

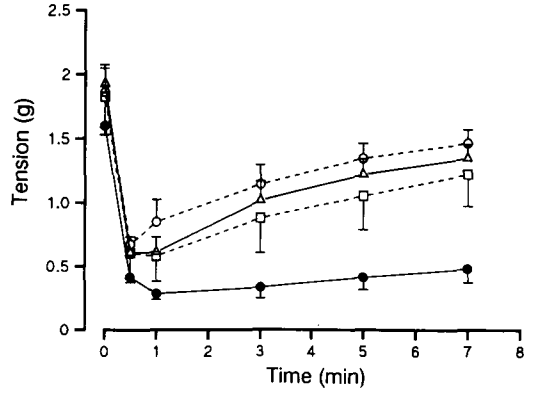

FIG. 2. Effect of EDTA (△, n = 4) and superoxide dismutase (□, n = 4) on the relaxing effect of carbachol (10 μM) on noradrenaline- (1 μM, ○, n = 4) precontracted rat aorta. Control preparations were precontracted with phenylephrine (1 μM, ●, n = 12). Mean ± s.e.m. (n = number of experiments.)

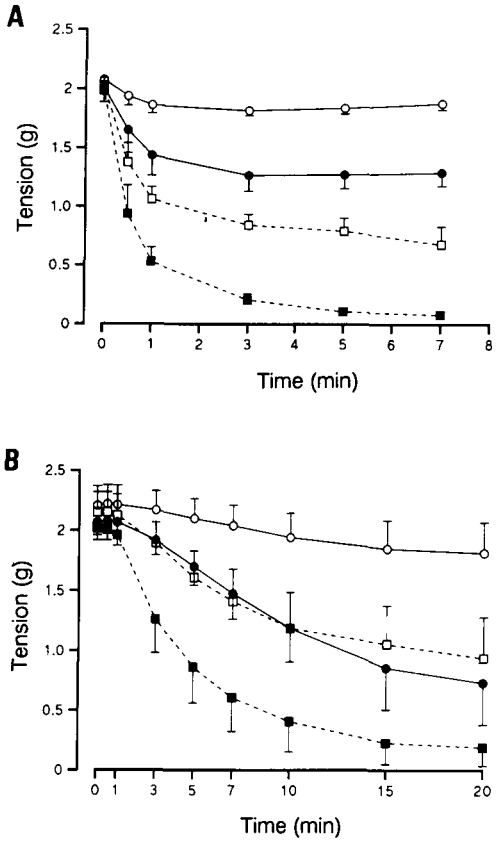

FIG. 3. A. Relaxing effect of sodium nitroprusside (0·01 μM ○, ●, n = 4; 0·1 μM □, ■, n = 4) on rat aorta precontracted with noradrenaline (1 μM ○, □) or phenylephrine (1 μM ●, ■). B. Relaxing effect of 8-Br-cGMP (100 μM ○, ●, n = 4; 300 μM □, ■, n = 4) on rat aorta precontracted with noradrenaline (1 μM ○, □) or phenylephrine (1 μM ●, ■). Mean ± s.e.m. (n = number of experiments.)

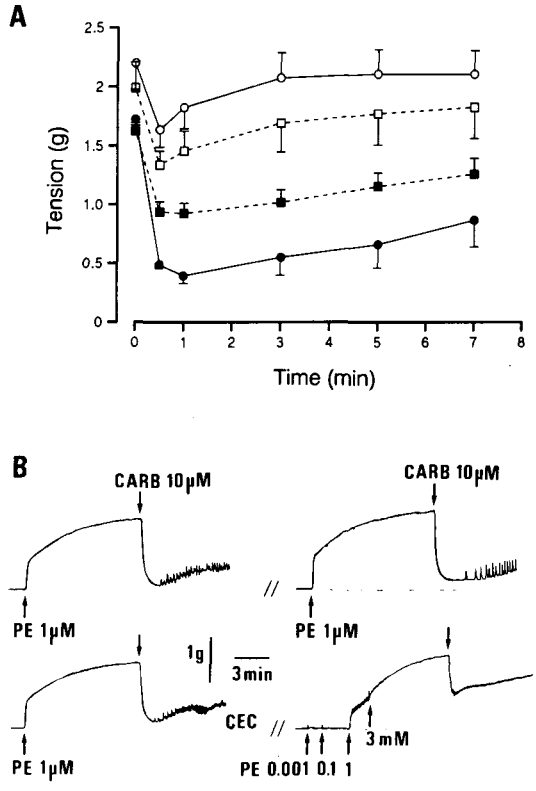

FIG. 4. A. Relaxing effect of carbachol (10 μM) added to the organ bath at t = 0 min on rat aorta precontracted with noradrenaline (○, □) or phenylephrine (●, ■) 1 μM in control preparations (○, ●, n = 4) or 1 mM for noradrenaline and 3 mM for phenylephrine after treatment with chloroethylclonidine (50 μM, □, ■, n = 4). (n = number of experiments.) B. Representative recordings of the relaxing effect of carbachol on phenylephrine precontracted aorta before and after chloroethylclonidine treatment. The first ring shows the reproducibility of carbachol-induced relaxation in phenylephrine- (1 μM) contracted preparations.

Discussion

The present report demonstrates that rings of rat aorta with an intact endothelium show a reduced relaxation response to carbachol when precontracted with noradrenaline, in comparison with phenylephrine. However, duration rather than intensity is affected in this respect and the phenomenon is fully demonstrated by the use of a single dose of carbachol observed during a relatively long period. By contrast, in most experiments, a fast dose-response curve is generally effected to test the relaxing properties of muscarinic agonists.

Phenylephrine, a preferential α_1-adrenoceptor agonist, and noradrenaline, a mixed α_1/α_2 agonist, are both full agonists in contracting rat aorta, and in this respect, noradrenaline is about 2- to 10-fold more sensitive than phenylephrine (Digges & Summers 1983; Hamed et al 1983; Macia et al 1984). However, the discriminative effect of EDRF is unlikely to be due to the difference in sensitivity between these two compounds since this occurs at the same tension and even when the concentration of phenylephrine is 10 times that of noradrenaline. In addition, a discrepancy in neuronal uptake or β-adrenergic activity between the compounds could be ruled out since rat aorta is sparsely innervated (Fleisch 1974) and β-adrenergic stimulation does not modulate noradrenaline-induced constriction in this artery (Cohen & Wiley 1977).

Noradrenaline is a dihydroxyphenyl derivative whereas phenylephrine has only a single hydroxy substitute, and thus, is more sensitive to autoxidation. This autoxidation of the catecholamine produces superoxide radicals which may react with nitric oxide. In fact, other polyhydroxyphenyl derivatives such as pyrogallol, trihydroxybenzene, may generate superoxide radicals (Marklund & Marklund 1974) and thus be potent inhibitors of EDRF (Moncada et al 1986). The effects of pyrogallol are reduced by superoxide dismutase (Moncada et al 1986; Ignarro et al 1988). Thus a discrepancy between the autoxidative properties of noradrenaline and phenylephrine may explain the variation of carbachol-induced relaxation observed in the present study. However, this is unlikely since neither EDTA nor superoxide dismutase were able to improve the relaxing action of carbachol in noradrenaline precontracted rings. In addition, an impairment of vasorelaxation is also observed with the two endothelium-independent agents sodium nitroprusside and 8-Br-cGMP when arteries are precontracted with noradrenaline in comparison with phenylephrine. Thus, a difference in the contractile properties may be hypothesized in order to explain the less pronounced effect of guanylate activation in noradrenaline-precontracted rings.

More recent studies have indicated that the α-adrenoceptor receptors of rat aorta are not a homogenous population (Han et al 1990; Oriowo & Bevan 1990; Piascik et al 1990). From these results it has been postulated that α-adrenoceptors of rat aorta are composed of an α_{1A}-subtype insensitive to alkylation by

chloroethylclonidine, and of an α_{1B}-subtype inactivated by chloroethylclonidine. The data of the present study indicate that pretreatment with chloroethylclonidine reduced the relaxing effects of carbachol when phenylephrine was used as a precontracting agent but had no effect when noradrenaline was used as the agonist. This result may indicate that EDRF is a more effective inhibitor of contraction induced by α_{1B}-adrenoceptor stimulation and that phenylephrine preferentially activates the α_{1B}-adrenoceptor subtype whereas noradrenaline is more sensitive on the α_{1A}-subtype. Several lines of evidence indicate that the two α_1-adrenoceptor subtypes induce contraction through different mechanisms. In fact, contractile processes induced by α_{1A}-subtypes are largely dependent on Ca^{2+} influx through potential-dependent channels, sensitive to calcium channel blockers (Han et al 1987, 1990). Among the contractile events triggered by α-adrenoceptor agonists such as intracellular calcium release, extracellular calcium influx through receptor linked channels and potential-dependent channels, the latter is less sensitive to the relaxant properties of EDRF (Auguet et al 1989a, b).

In conclusion, our results indicate a possible discriminative property of EDRF between α_1-adrenoceptor subtypes in rat aorta. A modification in the activity or proportion of these two α_1-adrenoceptor subtypes in pathological conditions such as hypertension may partially explain the diminished effectiveness of agonist-induced endothelium-dependent relaxation found in spontaneously hypertensive rat aorta (Konishi & Su 1983; Sim & Chua 1985).

References

Auguet, M., Delaflotte, S., Braquet, P. (1989a) Increased influence of endothelium in obese Zucker rat aorta. J. Pharm. Pharmacol. 41: 861–864

Auguet, M., Delaflotte, S., Pirotzky, E., Clostre, F., Braquet, P. (1989b) Role of endothelium on phenylephrine-triggered contractile events in aorta of spontaneously hypertensive rats. Fundam. Clin. Pharmacol. 3: 655–664

Cohen, M. L., Wiley, K. S. (1977) Specific enhancement of norepinephrine-induced contraction in rat veins after beta adrenergic antagonists. J. Pharmacol. Exp. Ther. 201: 406–416

Digges, K. G., Summers, R. J. (1983) Characterization of postsynaptic α-adrenoceptors in rat aortic strips and portal veins. Br. J. Pharmacol. 79: 655–665

Egleme, C., Godfraind, T., Miller, R. C. (1984) Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. Ibid. 81: 16–18

Fleisch, J. H. (1974) Pharmacology of the aorta—a brief review. Blood Vessel. 11: 193–211

Furchgott, R. F., Cherry, P. D. (1984) The muscarinic receptor of vascular endothelium that subserves vasodilation. Trends Pharmacol. Sci. 2: 45–48

Furchgott, R. F., Zawadzki, J. V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature (London) 288: 373–376

Furchgott, R. F., Zawadzki, J. V., Cherry, P. D. (1981) Role of endothelium in the vasodilator response to acetylcholine. In: Vanhoutte, P. M., Leusen, I. (eds) Vasodilatation. Raven Press, New York, pp 49–72

Hamed, A. T., Johnson, T. D., Charlton, K. G., Clarke, D. E. (1983) Pharmacological characterization of α-adrenoreceptor subtypes in rat isolated thoracic aorta. J. Auton. Pharmacol. 3: 265–273

Han, C., Abel, P. W., Minneman, K. P. (1987) Heterogeneity of alpha-1-adrenergic receptors revealed by chloroethylclonidine. Mol. Pharmacol. 36: 505–510

Han, C., Li, J., Minneman, K. P. (1990) Subtypes of α_1-adrenoceptors in rat blood vessels. Eur. J. Pharmacol. 190: 97–104

Ignarro, L. J., Byrns, R. E., Buga, G. M., Wood, G. M., Wood, K. S., Chaudhuri, G. (1988) Pharmacological evidence that endothelium-derived relaxing factor is nitric oxide: use of pyrogallol and superoxide dismutase to study endothelium-dependent and nitric oxide elicited vascular smooth muscle relaxation. J. Pharmacol. Exp. Ther. 244: 181–189

Johnson, R. D., Minneman, K. P. (1987) Differentiation of alpha-1-adrenergic receptors linked to phosphatidylinositol turnover and cyclic AMP accumulation in rat brain. Mol. Pharmacol. 31: 239–246

Konishi, M., Su, C. (1983) Role of endothelium in dilator responses of spontaneously hypertensive rat arteries. Hypertension 5: 881–886

Lues, I., Schümann, H. J. (1984) Effect of removing the endothelial cells on the reactivity of rat aortic segments to different α-adrenoceptor agonists. Naunyn Schmiedebergs Arch. Pharmacol. 328: 160–163

Macia, R. A., Matthews, W. D., Lafferty, J., DeMarinis, R. M. (1984) Assessment of alpha-adrenergic receptor subtypes in isolated rat aortic segments. Ibid. 325: 306–309

Marklund, S., Marklund, G. (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47: 469–474

Martin, W., Furchgott, R. F., Villani, G. M., Jothianandan, D. (1986) Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. J. Pharmacol. Exp. Ther. 237: 529–538

Moncada, S., Palmer, R. M. J., Gryglewski, R. (1986) Mechanism of action of some inhibitors of endothelium-derived relaxing factor. Proc. Natl. Acad. Sci. USA 83: 9164–9168

Morrow, A. L., Creese, I. (1986) Characterization of alpha-1-adrenergic receptor subtypes in rat brain: a reevaluation of [^{3}H]WB4104 and [^{3}H] prazosin binding. Mol. Pharmacol. 29: 321–330

Oriowo, M. A., Bevan, J. A. (1990) Chloroethylclonidine unmasks a non α-adrenoceptor noradrenaline binding site in the rat aorta. Eur. J. Pharmacol. 178: 243–246

Palmer, R. M. J., Ferrige, A. G., Moncada, S. (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327: 524–526

Piascik, M. T., Butler, B. T., Pruitt, T. A., Kusiak, J. W. (1990) Agonist interaction with alkylation-sensitive and resistant alpha-1-adrenoceptor subtypes J. Pharmacol. Exp. Ther. 254: 982–991

Rapoport, R. M., Draznin, M. B., Murad, F. (1983) Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. Nature 306: 174–176

Sim, M. K., Chua, M. E. (1985) Altered responsiveness of the aorta of hypertensive rats to histamine and acetylcholine. Jpn. J. Pharmacol. 39: 551–553