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α_2-Adrenoceptors and endothelium-dependent relaxation in canine large arteries

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1 Ring preparations from the carotid, coronary, renal, mesenteric and femoral arteries of the dog were precontracted with the thromboxane mimetic U46619, after ensuring that the resting conditions were comparable from the Laplace relationship.

2 In the presence of prazosin (1 μM) and propranolol (3 μM), noradrenaline (NA) relaxed the arteries in the order coronary > carotid > femoral > renal = mesenteric. When maximum relaxation to nitroglycerin (10 μM) was taken to be 100% the maximum relaxation to noradrenaline in each artery was: coronary 70%; carotid 34%; femoral 19%; renal 7% and mesenteric 2%.

3 In endothelium-intact arteries UK14304 mimicked the relaxation responses to NA and idazoxan shifted the curves to both agonists to the right, consistent with an α_2-adrenoceptor classification.

4 Substance P relaxed the arteries in the same order as for NA but showed higher efficacy i.e.: coronary 100%; carotid 80%; femoral 71% renal 49%; and mesenteric 41%. Removal of the endothelium abolished the relaxation to NA.

5 We conclude that endothelium-dependent relaxation to NA and substance P varies greatly across 5 large arteries of the dog. This may indicate that endothelium-derived relaxing factor (EDRF) release is site-dependent or that the efficacy of EDRF on smooth muscle varies; being greatest in the coronary and weakest in the renal and mesenteric arteries.

Introduction

Endothelial cells on arteries respond to a wide variety of vasodilator agents by releasing an endothelium-derived relaxing factor (EDRF) (Furchgott & Zawadzki, 1980; Furchgott, 1983). In both the dog and pig coronary artery, the constrictor amines, noradrenaline and 5-hydroxytryptamine caused relaxation under some conditions but only when the endothelium was intact. The receptors on endothelium mediating the response to noradrenaline were tentatively classified as α_2-adrenoceptors (Cocks & Angus, 1983; Angus *et al.*, 1986).

In this study we have examined the coronary, carotid, femoral, renal and mesenteric large arteries from the dog to determine the variation in endothelial cell-mediated relaxation from α_2-adrenoceptor activation. We have also compared the relaxation responses to the endothelium-dependent agent, substance P and endothelium-independent agent, nitroglycerin in the same vessels. Careful attention was given to the passive-force applied to the different arteries to ensure that the resting conditions were comparable. Our results demonstrate that there is a marked variation in the degree of relaxation mediated by α_2-adrenoceptors on endothelium of these large vessels ranging from approximately 70% on coronary arteries to nearly zero on renal and mesenteric arteries.

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Methods

General

Twenty-four greyhound dogs and four mongrel dogs of either sex weighing 15–32 kg were anaesthetized with sodium pentobarbitone 40 mg kg^{-1} i.v. The heart and lengths of the common carotid, cranial mesenteric, renal and femoral arteries were removed and placed in cold, oxygenated Krebs solution. The circumflex coronary artery was dissected free for a length of about 7 cm. All arteries had their connective tissue removed and were cut transversely into precise ring lengths of 4.22 mm by a fixed double bladed scalpel. The arteries were stored at room temperature in gassed Krebs solution for use later that day or were immediat-

ly suspended on wire hooks, in a 25 ml jacketed organ bath maintained at 37 ± 0.1°C. Hooks were fashioned from a length of surgical steel suture (Ethicon, BαS20). One hook was suspended from a Grass FT03C transducer. Springs were inserted either side of the transducer arm to maintain isometric contraction measurements against large forces as instructed by the manufacturer. The lower hook was fixed to a plastic (methacrylate) support leg attached to a micrometer (Mitutoyo, Tokyo, Japan). Force was recorded on single channel flat bed recorders (Rikadenki, Japan). Six such organ bath arrangements were run concurrently.

The Krebs solution had the following composition (mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2, glucose 11, EDTA 0.27 aerated with a gas mixture of 95% O_2 and 5% CO_2.

Passive force-diameter relationship

Each ring segment was equilibrated unstretched on wire hooks for 30 min. A set procedure was then followed to ensure that each ring segment was set at a passive tension equivalent to 90% of the internal circumference of that artery if it had been relaxed and perfused with a transmural pressure of 100 mmHg. This procedure was developed initially by Mulvany & Halpern (1976) for small arteries 150–200 μm internal diameter suspended as short ring segments on a myograph (Mulvany & Halpern, 1977). The following is a summary of the technique adapted here for large arteries.

If g is length of vessel (4.22 mm) and the wires of diameter d are separated from each other's inner surfaces by f mm then L, the internal circumference is given by:

$$L = (\pi + 2)d + 2f \quad (1)$$

This relationship holds since when stretched the artery is flat between the wires. The circumferential wall force (F) per unit length is the wall tension T (Figure 1).

$$T = F/2g \quad (2)$$

The measurements of F and T are made by first bringing the wires together (f = 0) and recording the micrometer reading. The wires are then separated in steps at one minute intervals by micrometer advancement. The force is recorded at the end of each interval together with the micrometer reading (Figure 2). These values of F_i and f_i (for ith step) are used to calculate L_i and T_i according to equations 1 and 2 above. T_i and L_i values are fitted by an exponential equation:

$$T_i = A \exp B L_i \quad (3)$$

The next step is to determine the internal circumference (L) for that vessel when relaxed and distended

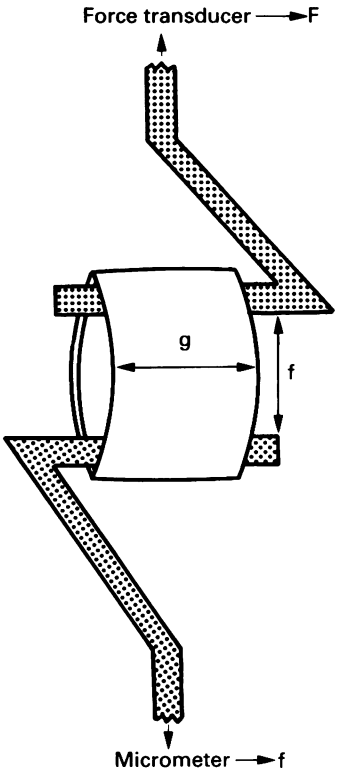

Figure 1 Schematic diagram of the technique used to support the artery ring *in vitro* and for the measurement of the passive force-internal circumference relationship.

by 100 mmHg transmural pressure (termed L_{100}).

$$\text{The isobar } T = r \times 100 \text{ mmHg} \quad (4)$$

is derived from the Laplace relationship:

$$P = 2\pi T/L \quad (5)$$

where P is the transmural pressure assuming (a) a thin walled tube and (b) that P is unaffected by the curvature caused by the suspending wires and r is the internal radius. A computer iterative fitting technique can readily determine the intersection of the isobar and exponential line and give the internal circumference L_{100} equivalent to a transmural pressure of 100 mmHg. It is then a simple task to determine the value of 0.9 L_{100} and to calculate the equivalent wall tension from equation 3 and thus the transmural pressure from equation 5. Finally the computer displays the micrometer setting necessary to place the

artery at 0.9 L_{100}. The circumferential isometric force at this setting was termed the passive or resting force (see Figure 2a). All calculations were accomplished readily with a Texas T159 card programmable calculator. Previous experiments in microvessels from rats have shown that maximum active tension was generated when vessels were stretched to a circumference of 0.9L_{100} (Mulvany & Halpern, 1977). Similarly, large femoral arteries developed their maximum force during depolarization with K^+ at a resting force equivalent to those about the 0.9 L_{100} mark i.e. 10–30 g.

Protocol

The ring segments were left for 30 min after the initial passive diameter-tension relationship had been established and the artery segment set to an internal circumference at 0.9 × L_{100}. Then a cumulative (0.5 log unit) concentration-response curve was obtained to a thromboxane A_2 mimetic, U46619. Only one concentration-response curve was obtained in each ring. From this first series of experiments the concentration of U46619 that gave 80% of the maximum contraction (EC_{80}) was determined from logistic curve fitting analysis (see below). In subsequent experiments, U46619 was applied at EC_{80} in the presence of α_1- and β-adrenoceptor blockade with prazosin (1 μM) and propranolol (3 μM). After the contraction had reached a plateau (approximately 10–15 min), concentration-response curves to noradrenaline or the α_2-adrenoceptor agonist UK14304 were constructed until maximum relaxation had occurred. To determine whether the artery could relax further to an endothelium-dependent dilator, a high concentration of substance P (10 nM) was given followed by nitroglycerin (10 μM). Finally, separate rings were again contracted to U46619 (EC_{80}) in the presence or absence of idazoxan (RX781094; 1 μM) and prazosin (1 μM) and propranolol (3 μM). Relaxation curves were obtained to noradrenaline and UK14304.

Statistical analysis

Responses were analysed in two ways. The full concentration-response curves to U46619 and the concentration-response curves for noradrenaline- and UK14304-induced relaxation were fitted to a logistic equation ($E = MA^P/A^P + K^P$) for each ring segment where E is response, M is maximum response, A is agonist concentration, K is EC_{50} and P is the slope parameter (Nakashima *et al.*, 1982). From this relationship computer estimates were determined of the concentrations required to give 10, 30, 50, 70 and 90% of the maximum response. These EC_{10-90} values were averaged for a number of rings and the mean and s.e.mean calculated. When full curves could not be established, responses to fixed concentrations were averaged and s.e.mean values calculated. In some instances the average s.e.mean within a ring for a concentration-response curve was calculated from two-way analysis of variance as (error mean square/number of animals)$^{0.5}$ after substracting the sums of squares 'between rings' and 'between contractions' from the total sums of squares. Two-tailed paired *t* tests were used to test statistical significance within rings and unpaired *t* tests for data between rings. Statistical significance in all tests was accepted at $P < 0.05$. The degree of displacement of the concentration-response curves in the presence of idazoxan (Figure 7) was estimated by using symmetrical (3 + 3) dose parallel line assay analysis for estimating dose-ratios (Colquhoun, 1971). For multiple comparisons of mean values (i.e. pressure values P in Table 1), one way analysis of variance was used and the modified *t* statistic calculated for the Bonferroni procedure (Wallenstein *et al.*, 1980).

Drugs

Drugs used and their sources were: U46619 ((1,5,5)-hydroxy-11α, 9α-(epoxymethano) prosta-5Z, 13E-dienoic acid, Upjohn, U.S.A.), UK14304 (5-bromo-b[2-imidazolin-2-yl-amino]-quinoxaline, Pfizer, U.K.), (±)-propranolol hydrochloride (ICI, U.K.), idazoxan (RX781094, Reckitt & Coleman, U.K.), (−)-noradrenaline bitartrate, (−)-isoprenaline bitartrate and substance P (Sigma, U.S.A.), nitroglycerin (Pohl Boskamp, West Germany), prazosin hydrochloride (Pfizer, U.S.A.). Drugs were dissolved in distilled water except noradrenaline which was dissolved in ascorbic acid (0.1 mM).

Results

Comparison of arteries

Representative traces from the initial passive stretch/force relationship are shown for a coronary and mesenteric artery ring segment (Figure 2a). The graphical display of the wall tension and internal circumference relationship for these two arteries indicate the less compliant nature of the coronary and smaller vessel diameter compared with the mesenteric vessel (Figure 2b). The 5 vessels had significantly different internal diameters, the mean values ranging from 3.48 mm to 5.73 mm for the coronary and mesenteric arteries respectively (Table 1). Calculated transmural pressure (P) for the resting internal circumference at 0.9 L_{100} showed that the vessels were set to a standard degree of stretch equivalent to a blood pressure of 68.3 mmHg (P). This resting force (F) generally correlated with the vessel diameter (Table 1).

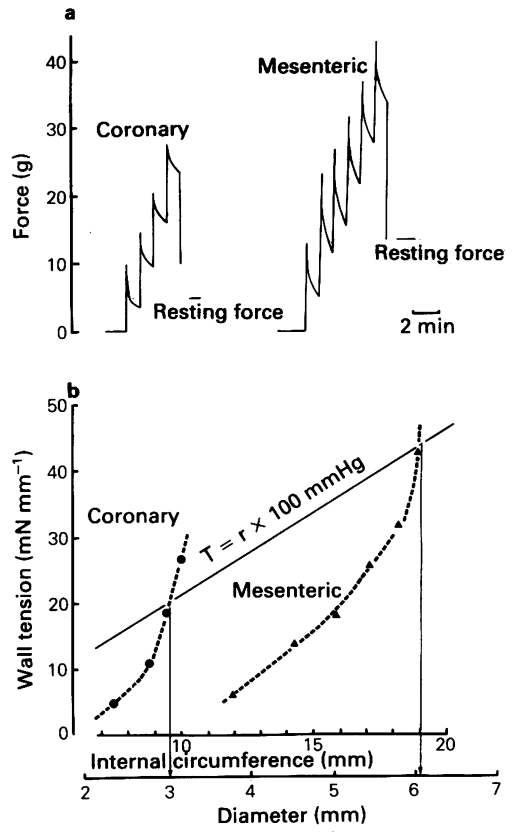

Figure 2 (a) Chart records (retouched for photographic clarity) of isometric force (ordinate scale) developed by a coronary and mesenteric artery ring during stepwise increase in diameter at one minute intervals. Resting force was set to the level indicated where the internal circumference is stretched to 0.9 times the circumference that would occur at a transmural pressure of 100 mmHg. (b) Graphical display of the values of wall tension ($mN\,mm^{-1}$) and internal circumference and diameter from the chart records (a). The isobar relating wall tension to radius from the Laplace relationship is indicated. The internal circumference and diameter for both coronary and mesenteric artery rings at an equivalent transmural pressure of 100 mm Hg are shown by the vertical arrows where the isobar and exponential curve intersect.

Contractile responses to U46619

The mesenteric and renal arteries were contracted to a significantly greater maximum force than the femoral, coronary or carotid arteries (Figure 3). In addition, the sensitivity to U46619 varied by more than 10 fold. The EC_{50} values ($-\log M \pm$ s.e.mean, $n = 6$) for U46619 in the different arteries were mesenteric 8.08 ± 0.07; renal 7.83 ± 0.11; coronary 7.58 ± 0.13; femoral 7.39 ± 0.09; and carotid 7.06 ± 0.04. EC_{80} concentrations for U46619 were as follows: mesenteric and renal 30 nM; coronary and femoral 100 nM and carotid artery 300 nM.

Relaxation responses to noradrenaline

Artery rings were precontracted by a concentration of U46619 (EC_{80}) determined from the previous experiments. In the presence of prazosin (1 μM) and propranolol (3 μM), noradrenaline relaxed some of the arteries in a concentration-dependent manner. The maximum relaxation, expressed as a percentage of the contracted force, was greatest for coronary arteries followed by carotid and femoral arteries (Figure 3). Invariably both the renal and mesenteric vessels failed to relax to noradrenaline under these conditions (Figure 3). Evidence that arteries had endothelium present and that they could relax to another endothelium-dependent vasodilator agonist, under these conditions of contraction by U46619, was shown by the response to a high concentration of substance P (10 nM).

The average responses to noradrenaline 10 μM, substance P (10 nM) and finally nitroglycerin (10 μM) were expressed as force (g) and as a percentage of the U46619-induced contraction. The coronary arteries were most effectively relaxed by noradrenaline and the addition of substance P relaxed these vessels completely. This was not the case for the other vessels. Generally, the poor response to noradrenaline was reflected in a similar pattern of response to substance P. Further addition of nitroglycerin sharply relaxed all the vessels to more than 75% of the contracted tone.

Removal of endothelium

Rings from the carotid, coronary and femoral arteries were either placed directly on the wires (endothelium intact, +E) or were rubbed gently on the luminal surface with a Krebs moistened filter paper taper to remove endothelium (−E). There was no significant difference between the contractions to U46619 of separate rings taken from within the same artery in the absence (−E) or presence (+E) of endothelium (unpaired t test) (Table 2). The resting force at circumference $0.9L_{100}$ was significantly lower for the carotid and coronary arteries with endothelium removed, compared with corresponding arteries with endothelium intact. This may have been partly due to the somewhat smaller diameters of the rings without endothelium.

In the presence of α_1- and β-adrenoceptor antagonists, noradrenaline relaxed the coronary, carotid and

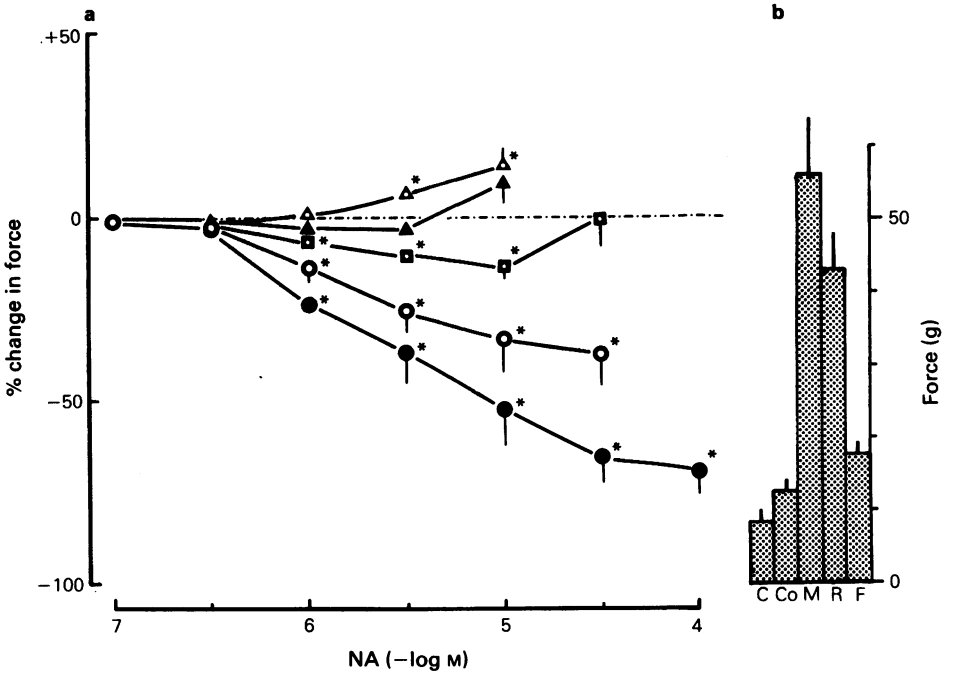

Figure 3 (a) Responses to noradrenaline (NA) in 5 different arteries precontracted with U46619 (EC_{80}). Data were calculated as % change in force from contracted value to U46619(g). (b) Illustrates the contraction to U46619(g) EC_{80}. Arteries were: M, mesenteric (Δ); R, renal (▲); F, femoral (□); C, carotid (○); Co, coronary (●). Each point represents the mean with vertical lines indicating s.e.mean (when larger than symbol), from 6 rings. *Represents values significantly different from starting contractile force.

femoral arteries only when endothelium was present. In the absence of endothelium, noradrenaline contracted the carotid artery by as much as a further 50% of the U46619-induced contraction (Figure 4). In the coronary and femoral artery these contractions were generally less than 10% (Figure 4). Evidence that endothelium had been successfully removed was shown by the failure of substance P to relax the vessel (Figure 4). Finally, the addition of nitroglycerin (10 μM) to each ring caused maximum or near maximum relaxation to occur whether or not endothelium was present (Figure 4).

Role of β-adrenoceptors

In the absence of propranolol, isoprenaline was a weak vasodilator of a U46619-contracted femoral artery such that isoprenaline 100 μM relaxed the vessel by less

Table 1 A summary of the vessel parameters

Artery	n	*D* (mm)	*P* (mmHg)	*F* (g)	n	*U46619*
Coronary	14	3.48 ± 0.12	65.2 ± 1.8	8.74 ± 0.64	8	12.3 ± 1.4
Carotid	14	4.20 ± 0.12	70.9 ± 1.1	10.23 ± 0.33	8	8.4 ± 1.7
Renal	12	4.52 ± 0.24	73.6 ± 3.0	10.73 ± 0.65	6	43.0 ± 5.4
Femoral	14	4.84 ± 0.24	66.1 ± 2.0	12.41 ± 1.02	8	18.0 ± 2.1
Mesenteric	12	5.73 ± 0.19	66.0 ± 2.3	11.46 ± 0.71	6	56.3 ± 7.8

Abbreviations used: *n*, number of rings; D, internal diameter (mm) estimated for transmural pressure of 100 mmHg; P, equivalent transmural pressure needed to distend artery at 90% of internal circumference at 100 mmHg (i.e. $0.9L_{100}$); F, isometric force recorded at $0.9L_{100}$. U46619, increase in force at maximum response in a full concentration-response curve to U46619.

Table 2 Effect of endothelium removal on vessel parameters

Artery	*E*	n	*D* (mm)	*P* (mmHg)	*Passive F* (g)	*Active F* (g)
Carotid	+	8	4.15 ± 0.16	72.3 ± 1.5	10.24 ± 0.36	8.38 ± 1.65
	−	5	3.65 ± 0.20	75.1 ± 3.2	4.94 ± 0.44*	5.76 ± 1.30
Coronary	+	8	3.49 ± 0.14	62.4 ± 2.6	8.53 ± 0.70	12.30 ± 1.36
	−	8	3.08 ± 0.22	69.4 ± 3.2	6.30 ± 0.70*	10.50 ± 1.52
Femoral	+	8	4.36 ± 0.29	66.1 ± 2.0	11.26 ± 1.57	17.98 ± 2.07
	−	5	4.25 ± 0.37	67.6 ± 1.0	10.36 ± 1.71	16.84 ± 1.96

Abbreviations used: E, endothelium present (+) or absent (−); *n*, number of rings; D, internal diameter estimated at transmural pressure of 100 mmHg; P, equivalent transmural pressure needed to distend artery at 90% of internal circumference at 100 mmHg (i.e. $0.9L_{100}$); passive F, resting force at $0.9L_{100}$; active F, increase in force from passive force caused by U46619 at EC_{80}. *Indicates significant difference between E+ and E− rings ($P < 0.05$, unpaired *t* test).

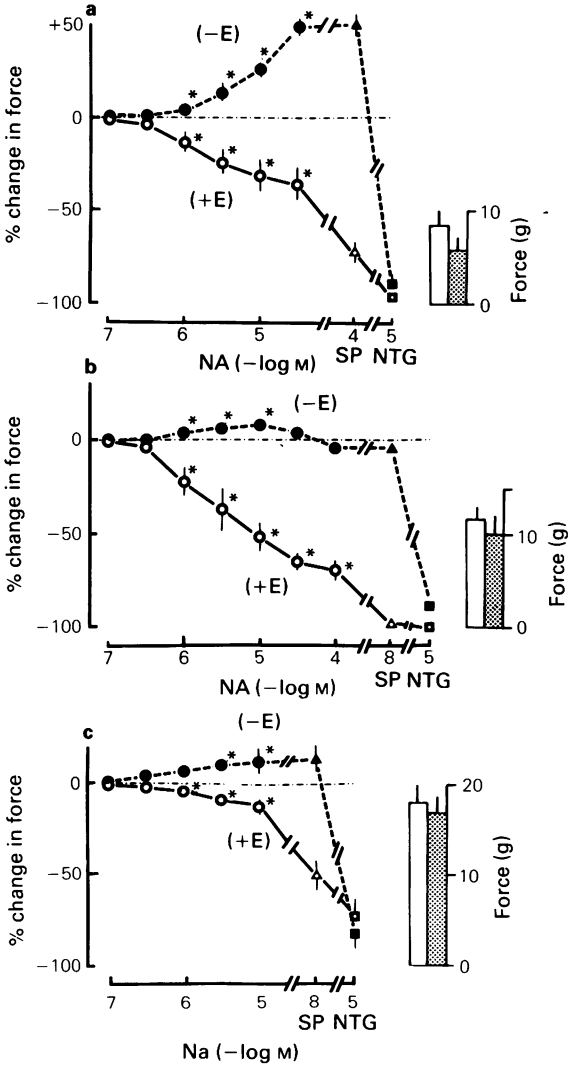

Figure 4 Concentration-response curves for noradrenaline (NA) in the absence (− E) and presence (+ E) of endothelium in carotid (a), coronary (b) and femoral (c) arteries after contraction with U46619 (EC_{80}) in the presence of prazosin and propranolol. Ordinate scale: changes in force expressed as % of U46619-induced contraction. Responses after substance P (SP) and nitroglycerin (NTG) are also shown. Histograms illustrate the contractions to U46619(g) of arteries in the presence (open columns) and absence (stippled columns) of intact endothelium.

than 50% (Figure 5). In the presence of this high concentration of β-agonist, noradrenaline 1–30 μM relaxed the artery still further. Endothelium was present as shown by the response to substance P. In a second artery ring, the response to isoprenaline (up to 100 μM) was almost completely abolished by propranolol (3 μM). However, the addition of noradrenaline (1–30 μM) still relaxed the vessel, in this case to a somewhat greater extent than the group average, but more transiently. Clearly substantial β-adrenoceptor blockade had not altered the relaxation to noradrenaline (Figure 5).

α_2-Adrenoceptor classification

Comparative assays for noradrenaline and the α_2-adrenoceptor selective agonist, UK14304, were conducted in coronary artery rings. In the presence of propranolol (3 μM) and prazosin (1 μM), concentration-response curves were constructed to noradrenaline or UK14304 in the absence or presence of 1 μM idazoxan (RX781094). The artery rings were of similar size, and were set at similar wall tensions as judged by the transmural pressure (P) calculated at $0.9L_{100}$ (Table 3). The α_2-adrenoceptor agonist UK14304 was 27 fold more potent than noradrenaline ($EC_{50} = 7.01 \pm 0.06$ and 5.58 ± 0.15; $-\log M \pm 1$

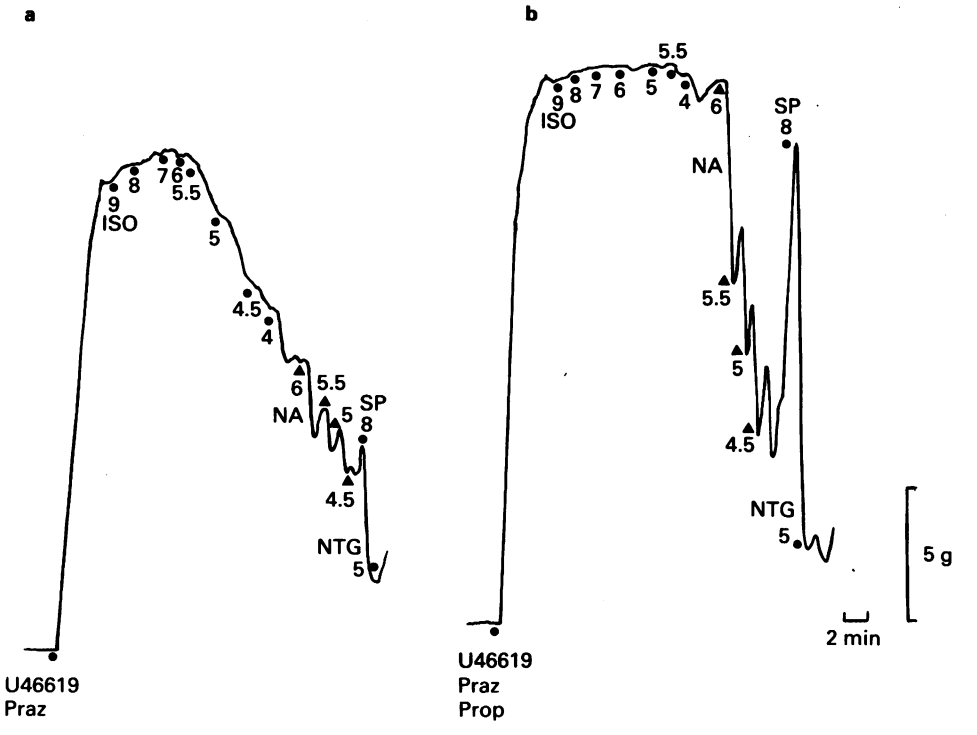

Figure 5 Representative chart records of the relaxation responses to isoprenaline (ISO) and noradrenaline (NA) in two greyhound femoral artery rings. (a) In the absence of propranolol and (b) in the presence of propranolol (Prop, 3 μM). Both rings were contracted with U46619 (EC_{80}) in the presence of prazosin (Praz, 1 μM). Responses to substance P (SP) and nitroglycerin (NTG) were tested as indicated. Numbers refer to concentrations (− log M).

s.e.mean respectively), but the maximum relaxation to noradrenaline was significantly greater (64.1 ± 5.2%) than that to UK14304 (48.4 ± 4.7%; $P<0.05$ d.f. = 12, unpaired t test). The relaxation to substance P was almost maximal in the two groups indicating that the response to another endothelium-dependent relaxant agonist was similar in the two assays (Figure 6). The α_2-adrenoceptor antagonist idazoxan shifted both the noradrenaline and UK14304 concentration-response curves to the right in parallel but did not alter the maximum response to either agonist or to substance P (Figure 7a,c). The symmetrical 3 + 3 analysis of the responses to noradrenaline gave a concentration-ratio of 5.60 (95% confidence limits 2.25, 13.09) with idazoxan 1 μM. This corresponds to a pK_B value, assuming competitive kinetics, of 6.66 (95% confidence limits 6.09, 7.08). In comparison, the UK14304 concentration-response curves gave a concentration-ratio of 29.5 (95% confidence limits 13.1, 74.11) corresponding to a pK_B of 7.45 (95% confidence limits 7.08, 7.86). Alternative assessment of the degree of antagonism was by comparing the EC_{50} values from the logistic fitted curves of both noradrenaline and UK14304 in the absence and presence of idazoxan (1 μM) (Figure 7b,d). These EC_{50} values gave concentration-ratios of 6.16 and 15.13 for noradrenaline and UK14304 respectively; equivalent to pK_B values of 6.71 and 7.15 for idazoxan. Thus both methods suggested a lower pK_B for idazoxan against noradrenaline compared with the agonist UK14304.

Discussion

In this work we found that α_2-adrenoceptors mediated an endothelium-dependent relaxation in the coronary, carotid and femoral large arteries of the dog, the magnitude of which was variable. In contrast, no significant α_2-adrenoceptor relaxation was observed in the mesenteric or renal arteries. We suggest that this variation is probably due to a non-uniform EDRF release or to an altered EDRF efficacy in different arteries.

Table 3 The parameters of coronary arteries used to evaluate the effects of noradrenaline and UK14304

Group	n	*D* (mm)	*P* (mmHg)	*Passive F*	*Active F*
Noradrenaline	14	3.20 ± 0.11	62.9 ± 1.8	7.38 ± 0.39	17.30 ± 1.79
UK14304	14	3.31 ± 0.12	62.9 ± 1.7	7.91 ± 0.35	14.45 ± 1.51

For abbreviations see Table 2.

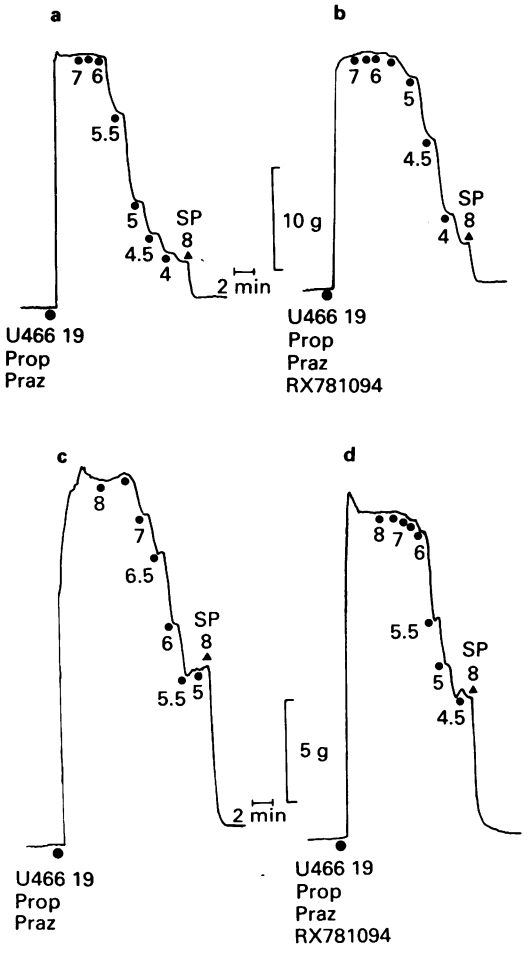

Figure 6 Representative chart records of concentration-response curves for the relaxation induced by noradrenaline (a,b) and UK14304 (c,d) of 4 coronary artery rings precontracted with U46619 in the presence of propranolol (3 μM) and prazosin (1 μM). (b and d) Experiments conducted in the presence of idazoxan (RX781094, 1 μM). Substance P (SP) was given at the end of each experiment. Numbers refer to concentrations (− log M).

Contraction conditions

Any comparison between arteries of different size and contractility should be set initially at the same passive conditions. The Mulvany-Halpern procedure seemed the most appropriate. Evidence that similar passive conditions were met in this study was that there was no significant difference between the passive pressures (P) estimated to be required to distend each artery to an internal circumference of 90% of that at 100 mmHg. To actively precontract the artery, the thromboxane A_2 mimetic U46619 was chosen since it gave sustained contractions of all large arteries for at least 3 h (Angus & Brazenor, 1983). Wide variation was observed in the sensitivity (EC_{50}) to U46619 and also in the level of maximum contraction. These features of the full concentration-response curves to U46619 confirmed the necessity to establish the contraction assays before proceeding with the dilator responses. For example although the resting passive force in the coronary (8.7 g) and mesenteric (11.5 g) arteries were reasonably comparable, the increases in force due to maximum concentrations of U46619 were 12.3 g and 56.3 g respectively. This raises the question as to whether an artery contracted to 56 g active force would relax as readily as an artery contracted to only 12 g by the same agent. In the present experiments, nitroglycerin relaxed coronary and carotid arteries to almost 100% of the U46619 contraction while in the femoral, renal and mesenteric arteries the relaxations were similar (75–80%) even though the EC_{80} U46619 contraction varied from 18 g for the femoral to 56.3 g for the mesenteric artery (Table 1). The maximum relaxation responses to nitroglycerin were also independent of endothelium and independent of whether the artery had been partly relaxed or further contracted with noradrenaline and substance P (Figure 4).

Comparison of noradrenaline and substance P

Nitroglycerin acts entirely independently of endothelium and stimulates guanosine 3':5'-cyclic monophosphate (cyclic GMP) formation in vascular smooth muscle (Rapoport & Murad, 1983). Similarly, endothelium-dependent dilator agonists such as substance P and acetylcholine that release EDRF also stimulate the production of cyclic GMP in smooth

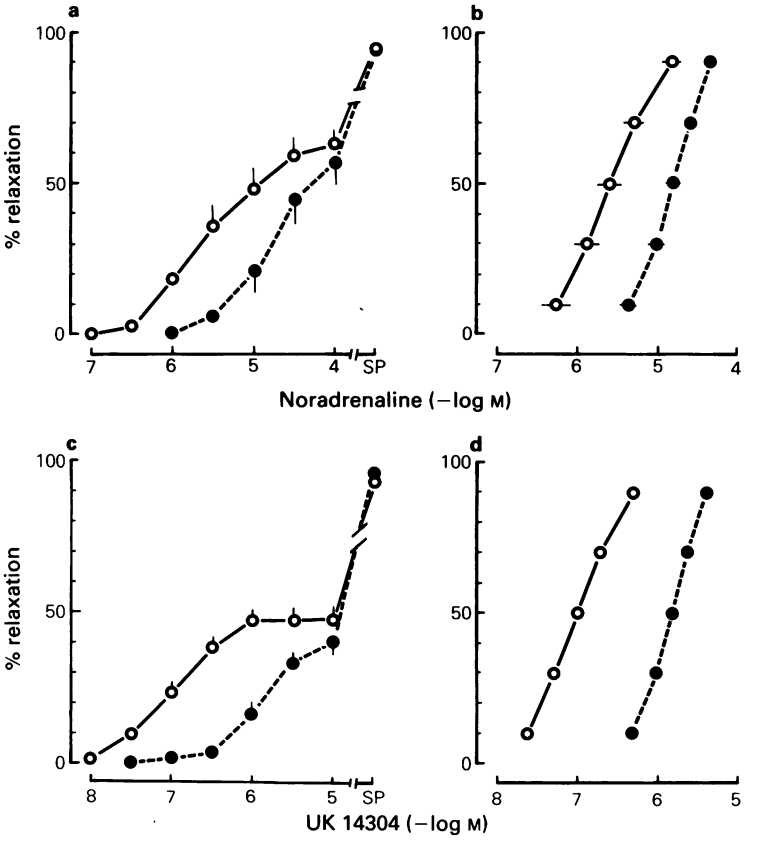

Figure 7 Concentration-response curves for noradrenaline (a,b) and UK14304 (c,d)-induced relaxation in the absence (O—O) and presence (●---●) of idazoxan (RX781094, 1 μM). (a and c) Points represent mean values, with vertical lines indicating 1 s.e.mean, of % relaxation of U46619-induced contraction. (b and d) Mean EC_{10}–EC_{90} values with horizontal lines indicating s.e.mean, determined from logistic fitting of individual concentration-response curves where 100% relaxation was considered as maximum response to noradrenaline or UK14304. Responses to substance P (SP) 10 nM, are also shown.

muscle (Rapoport & Murad, 1983). Therefore, it was considered that it might be feasible to use the maximum relaxation response to nitroglycerin as a marker for the maximum possible cyclic GMP-mediated response that could be expected from EDRF released by substance P or noradrenaline. Substance P maximally relaxed coronary arteries (100%) but was weaker on carotid (79.6%), femoral (71%), renal (48.7%) and mesenteric (40.6%) arteries when the response to nitroglycerin was taken as 100%. This order of activity of the relaxation to substance P was identical to that for noradrenaline (NA) on α_2-adrenoceptors. Thus a correlation graph of NA- and substance P-induced relaxation responses indicates that either the amount of EDRF released or the efficacy of EDRF on the smooth muscle is not uniform across the 5 vessels, being least on the mesenteric and strongest on the coronary artery. In addition, the correlation also suggests that substance P has a higher efficacy than noradrenaline since the correlation line was shifted to the right of the line of identity in Figure 8. This explanation does not take into account any α_2-adrenoceptor-mediated contraction of vascular smooth muscle which is quite variable and would functionally antagonize the α_2-mediated relaxation due to EDRF release. Thus the carotid artery contracts further to noradrenaline in the absence of endothelium, by as much as 50% (Figure 4). However, an α_2-adrenoceptor-mediated contraction does not explain the poor relaxation in the femoral, renal and mesenteric

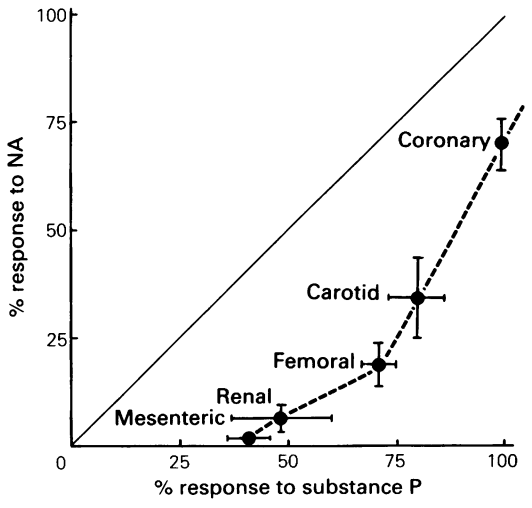

Figure 8 Correlation between relaxation responses to noradrenaline (NA, ordinate scale) and substance P (abscissa scale) in 5 different arteries. Responses are % of maximum relaxation to nitroglycerin measured in each ring segment (= 100%). Values represent means ± s.e.mean from 6–8 rings (some error bars are within the symbol).

arteries, since endothelium removal was not associated with large α_2-adrenoceptor-mediated contractions (i.e. Figure 4). The coronary artery stands out as the artery which is most responsive to EDRF released by either noradrenaline or substance P.

In our experiments relaxation responses to substance P and nitroglycerin were measured sequentially and in the presence of noradrenaline. Endothelium-dependent dilator agonists and nitroglycerin relax arterial rings promptly but constrictor tone readily returns even in the continued presence of a dilator agonist (see Figure 5b). If a second endothelium-dependent vasodilator agonist (i.e. substance P) is applied in the presence of noradrenaline, then the maximum response was similar to that observed when the constrictor tone had not been allowed to recover fully before applying substance P (see Figure 5a). Thus, although there may be some interaction between the two endothelium-dependent dilators in this assay, the use of maximum concentrations of substance P should have ensured that the relaxation was the peak obtainable EDRF response in each artery.

Classification of receptors as α_2-adrenoceptors

The experiments here support our original contention that the receptor to noradrenaline on endothelium is of the α_2-subtype (Cocks & Angus, 1983). The selective and potent α_2-adrenoceptor agonist UK14304 caused a maximum relaxation response that was less than that to noradrenaline, consistent with a lower efficacy. Furthermore idazoxan displaced both the noradrenaline and UK14304 concentration-response curves to the right and from a single concentration we estimated pK_B values of 6.7 and 7.5 for idazoxan against NA and UK14304, respectively. These are within the range (6.58–7.56) found for idazoxan in the rat tail artery (Medgett & Langer, 1984). The low value for noradrenaline may be related to removal, uptake or other sites of amine loss (Medgett & Langer, 1984). The failure of isoprenaline to relax the femoral artery in the presence of propranolol and the finding that the response to noradrenaline was unaffected by β-adrenoceptor blockade again supports the concept of α-adrenoceptors being the loci of activity. In addition, prazosin (1 μM) increased the relaxation response to noradrenaline, presumably by blocking the α_1-adrenoceptor-mediated contraction on smooth muscle. Indeed, in the absence of prazosin no relaxation response was observed to noradrenaline in the dog coronary artery (Angus *et al.*, 1986).

Role of α_2-adrenoceptors on endothelium

This work serves to illustrate another action of noradrenaline on the artery wall besides that on the smooth muscle cells. The endothelial cells participate readily by releasing EDRF, at least in the coronary artery and to a lesser extent in the carotid and femoral arteries. To illustrate the α_2-adrenoceptor-mediated activity directly, it was necessary to have intact endothelium, and α_1- and β-adrenoceptor antagonists present. The signal is quite powerful in some circumstances as it has been demonstrated that this EDRF release can alter the maximum response and the location of the concentration-response curves for the contractile effects of noradrenaline in dog and pig coronary arteries (Cocks & Angus, 1983). Just as we could not demonstrate α_2-adrenoceptor-mediated relaxation in dog renal or mesenteric vessels in our experiments, we have been unable to find any α_2-adrenoceptor-mediated relaxation of bovine coronary artery (Angus *et al.*, 1986). Yet, this latter artery is readily relaxed by bradykinin (Angus *et al.*, 1986). Thus, the location and effectiveness of α_2-adrenoceptor-mediated EDRF release varied between arteries within species, and does not hold for the coronary artery between species, making any speculation of a physiological role for this phenomenon hazardous.

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