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Influence of the vascular endothelium on agonist-induced contractions and relaxations in rat aorta

Gillian R. Bullock*, S.G. Taylor** & A.H. Weston**,[1]

Ciba Geigy Ltd*, CH-4002, Basle, Switzerland and Smooth Muscle Research Group**, Department of Physiological Sciences, Medical School, University of Manchester, Manchester M13 9PT

1 The influence of the vascular endothelium on agonist-induced contractions and relaxations has been measured using intact segments of rat aorta. Contiguous rubbed segments were used as controls.

2 Angiotensin II, histamine, noradrenaline, U46619 and UK14304 contracted both rubbed and intact tissues. The threshold spasmogenic concentrations of these agonists were lower in rubbed tissues than in intact preparations.

3 The sensitivity and responsiveness of tissues to angiotensin II, histamine, noradrenaline and UK14304 were greater in rubbed than in intact tissues.

4 Acetylcholine and histamine relaxed the established spasms of intact tissues but not those of rubbed preparations, These relaxant effects of acetylcholine were abolished by pre-incubation with haemoglobin.

5 In the presence of prazosin, noradrenaline or UK14304 relaxed established contractions in intact tissues. These effects were antagonized by idazoxan or by pre-incubation with haemoglobin.

6 In intact preparations, idazoxan had no effect on the spasmogenic sensitivity and responsiveness to UK14304.

7 Pre-incubation with haemoglobin augmented the spasmogenic actions of noradrenaline, U46619 or UK14304 in intact tissues, but had no effect on these responses in rubbed preparations.

8 Tissue concentrations of cyclic GMP were greater in intact than in rubbed tissues. A concentration of acetylcholine (10 μM) evoking just maximal mechanical inhibition produced a significant increase in cyclic GMP concentration in intact preparations. However, no detectable changes in cyclic GMP concentration were produced by UK14304 (10 μM) or by acetylcholine (30 nM), concentrations which were equi-effective in inhibiting mechanical activity.

9 In the presence of threshold spasmogenic concentrations of noradrenaline, the contractile effects of angiotensin II were augmented and became comparable to those observed in rubbed preparations. In the presence of greater concentrations of noradrenaline, angiotensin II always produced an additional contraction.

10 It is concluded that the presence of the vascular endothelium limits the spasmogenic action of a variety of agonists. Although spasmogens like noradrenaline and UK14304 can stimulate the release of endothelium-derived relaxing factor (EDRF) via α_2-adrenoceptors, the inhibitory effects of EDRF largely result from the spontaneous release of this substance.

Introduction

Since the initial work with acetylcholine on rabbit aorta (Furchgott & Zawadski, 1980), the inhibitory actions of several agents in a variety of vascular tissues have been shown to be completely or partially dependent on the presence of vascular endothelium. These inhibitory effects result from the stimulated release of an inhibitory substance, endothelium derived relaxing factor (EDRF, see Furchgott, 1984). Subsequently it has been demonstrated that the contractions induced by some spasmogens in rat aorta (Allan *et al.*, 1983; Fortes *et al.*, 1983; Eglème *et al.*, 1984; Miller *et al.*, 1984; Carrier & White, 1985; Godfraind *et al.*, 1985; Murakami *et al.*, 1985), dog and pig coronary arteries (Cocks & Angus, 1983; Angus *et al.*, 1986), dog pulmonary and systemic blood vessels (Miller & Vanhoutte, 1985) and some human isolated arteries

[1]Author for correspondence

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(Calvete *et al.*, 1984) are augmented by removal of the vascular endothelium from these tissues.

Recently, Malta *et al.* (1986) and Martin *et al.* (1986) have suggested that basal release of EDRF could largely account for the observed endothelium-dependent differences in the sensitivity and responsiveness of vascular tissues to spasmogens. In the present study, the effect of the endothelium in modulating a variety of agonist-induced responses in rat aorta has been examined, paying special attention to the role of basal and stimulated release of EDRF.

Methods

Male Wistar rats were decapitated, the thoracic aorta was exposed, cleared of surrounding tissue *in situ* and removed. The aorta was cut into four rings approximately 0.5 cm long and each was opened along its longitudinal axis to form a flat sheet. A tiny bent pin, with thread attached, was carefully pushed through each longitudinally-cut edge. The endothelium on two of the sheets was mechanically removed by rubbing with a moistened cotton bud to produce two matched pairs of rubbed/intact preparations. Each tissue was mounted isometrically under 1 g tension in a 20 ml tissue bath containing a bicarbonate-buffered physiological salt solution (PSS) at 37°C, gassed with 95% O_2 and 5% CO_2. After 30 min, the preparations were retensioned to 1 g and a further 1 h equilibration period was allowed before the start of an experiment. Tension changes were recorded using a multichannel potentiometric recorder.

Effect of the endothelium on spasmogenic responses

Cumulative concentration-effect experiments were carried out on matched pairs of rubbed/intact tissues using angiotensin II, histamine, noradrenaline, UK14304 and U46619. When the maximum tension development to any single agonist had been achieved, the ability of acetylcholine to relax the tissue was examined using a cumulative protocol as a functional test of endothelial integrity.

Agonist-induced inhibition of noradrenaline- and U46619-induced contractions

Cumulative concentration-effect experiments were performed on matched pairs of rubbed/intact tissues from the same aorta using noradrenaline (2 μM, an approximate E_{max}) to induce tension. In the continuing presence of noradrenaline (2 μM), cumulative concentration-effect experiments were performed using angiotensin II, histamine and UK14304. In a second series of experiments, intact tissues were contracted with a low concentration of noradrenaline (20 nM, an approximate EC_{30}) and cumulative concentration-effect experiments were performed using angiotensin II, histamine and UK14304. Matched pairs of rubbed/intact tissues were exposed to U46619 (20 nM) for 15 min, during which time the induced contraction developed fully. The inhibitory effects of acetylcholine, angiotension II, histamine, noradrenaline and UK14304 were then examined using a cumulative protocol.

Effects of adrenoceptor antagonists on inhibitory responses to UK14304 and noradrenaline

(1) *Effects of prazosin, propranolol and phentolamine* Matched pairs of rubbed/intact tissues were exposed to PSS containing prazosin (1 μM) for 30 min. They were then challenged with equi-effective spasmogenic concentrations of U46619 (10 nM and 20 nM respectively) in the continuing presence of prazosin. After 15 min when maximum tension development had occurred, the inhibitory effects of UK14304 were examined using a cumulative protocol. When the maximum inhibitory effects of this agent had been obtained, tissues were challenged with acetylcholine (10 μM) in the continuing presence of UK14304. Preparations were subsequently washed with PSS containing prazosin (1 μM) for 30 min after which they were additionally exposed to propranolol (1 μM) for 30 min. The inhibitory effects of UK14304 against U46619 contractions were re-tested followed by challenge with acetylcholine as already described. After washing with PSS containing prazosin and propranolol (each 1 μM) for 30 min, the tissues were additionally exposed to phentolamine (1 μM) for 30 min before re-examining the inhibitory effects of UK14304 and acetylcholine against U46619-induced contractions.

In a parallel series of experiments, the inhibitory effects of noradrenaline against U46619 contractions were examined in PSS containing prazosin and propranolol (each 1 μM).

(2) *Effects of idazoxan* In these experiments, intact tissues only were exposed to PSS containing prazosin (1 μM) and propranolol (1 μM) and challenged with U46619 (20 nM). After 15 min, the inhibitory effects of UK14304 and noradrenaline were examined. When the maximum inhibitory effects had been obtained the tissues were challenged with acetylcholine (10 μM) and then washed with PSS containing prazosin and propranolol (each 1 μM) for 30 min. To determine the potency of idazoxan, four concentration-effect experiments were performed on each strip of tissue, separated by a washout period of 1 h. The first such experiment served as control whereas the following ones were carried out in the presence of increasing concentrations of idazoxan (0.01, 0.1 or 1 μM) which

was added 30 min before exposure to U46619. The pA_2 value for idazoxan against UK14304 was calculated by the method of Arunlakshana & Schild (1959). In a separate series of experiments, matched pairs of rubbed/intact tissues were pretreated with idazoxan (0.1 or 1 μM) for 30 min and the effects of UK14304 examined in the continuing presence of idazoxan.

Effects of haemoglobin

Matched pairs of rubbed/intact preparations were exposed to haemoglobin (10 μM) for 5 min before cumulative concentration-effect experiments were carried out using U46619, noradrenaline and UK14304. In a second series of experiments, tissues were exposed to spasmogens and when the contraction had developed fully, were additionally exposed to haemoglobin (10 μM). After 5 min, tissues were exposed to inhibitory agents in the continuing presence of the spasmogen and haemoglobin.

To allow a comparison between endothelium-dependent inhibitory effects, control and test tissues were carefully matched so that the tension induced in the tissues was the same in the presence or absence of haemoglobin.

Determination of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) levels

Although rubbed aortic strips were used in this series of experiments, the intact tissues consisted of unopened aortic rings. Silver staining had shown that such unopened preparations maintained the integrity of the vascular endothelium better than opened strips. The anatomical position of each ring/strip was noted (upper, upper middle, lower middle, lower) before it was assigned using a balanced design to its respective group.

Each experimental group of tissues was equilibrated in 80 ml PSS maintained at 37°C and bubbled with 95% O_2 and 5% CO_2 for 90 min. During this time the PSS was changed once. Either acetylcholine (30 nM or 10 μM) or UK14304 (10 μM) was then added and after 30 s the tissues were plunged into liquid nitrogen. Control groups consisted of rubbed/intact tissues treated with vehicle only and rubbed/intact tissues pre-incubated with prazosin (1 μM) for the final 30 min of their 90 min equilibration period.

Tissues were thawed in 1 ml of 10% trichloroacetic acid and then each was homogenized separately with a Potter glass/glass homogeniser. The homogenate was centrifuged at 3,000 *g* for 15 min at 4°C and the supernatant was extracted 4 times with 3 volumes of water-saturated ether, discarding the ether phase each time. Residual ether was then removed using nitrogen gas. A portion of the extract was acetylated and assayed for cyclic GMP using a radioimmunoassay kit (NEN). Precipitates were solubilised in 1 ml 1 M NaOH and a portion was used for protein determination by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Cyclic nucleotide concentrations were expressed as pmol mg^{-1} protein.

Silver staining technique

A simple silver staining technique (Poole *et al.*, 1958) was used to verify the presence or absence of endothelium. At the end of an experiment, tissues were rinsed in distilled water and immediately placed for 5 min in a freshly-prepared 2% silver nitrate solution. The tissues were washed and kept moist with distilled water while the silver stain was developed using a fibre-optics light source. The tissues were then viewed by 100 × magnification under a binocular microscope.

Drugs and solutions

The bicarbonate-buffered physiological salt solution (PSS) used had the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11.

Acetylcholine chloride (Sigma) and phentolamine mesylate (Ciba) were prepared as stock solutions in absolute ethanol. Angiotensin II (Ciba), (−)-ascorbic acid (BDH), histamine hydrochloride (Sigma), idazoxan hydrochloride (Reckitt), propranolol hydrochloride (ICI), silver nitrate (BDH), U46619 (9, 11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$; Upjohn) and UK14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline; Pfizer) were dissolved in distilled water. (−)-Noradrenaline bitartrate monohydrate (Sigma) was prepared as a stock solution in 0.1 N hydrochloric acid. Prazosin hydrochloride (Pfizer) was prepared as a stock solution in 20% N, N-dimethyl acetamide (Sigma).

Ascorbic acid, 100 μM, was present in all experiments with noradrenaline and UK14304 to minimize their oxidation. Dilutions of all agents were prepared in double distilled water immediately before use and kept on melting ice. Haemoglobin (Sigma) was prepared by the method of Martin *et al.* (1985). Final bath concentrations of ethanol and N, N-dimethyl acetamide were 0.01% and 0.002%, respectively.

Data presentation and analysis

Absolute tension development was measured throughout so that comparisons could be made of both the sensitivity and responsiveness of rubbed and intact tissues. Relaxation responses were calculated as a percentage of the induced tension which existed at the start of a relaxant concentration-effect experiment. The agonist concentration which produced 50% of the maximal response for the agonist (EC_{50}) was derived

from the appropriate concentration-effect curve and expressed as the $\log_{10}$ value ± 95% confidence interval. Data are normally expressed as means ± s.e.mean. Tests of significance were made by use of Student's two-tailed unpaired t test.

Results

Effect of the endothelium on spasmogenic responses

Preliminary experiments suggested that both the responsiveness and the sensitivity of a tissue to a variety of spasmogens were greater in the absence of the vascular endothelium. The results of a formal comparison of responses to a variety of spasmogens elicited on matched pairs of rubbed and intact aortic segments are summarized in Table 1.The threshold concentration for all spasmogens was lower in the rubbed preparations (< 10% endothelial cover) than in those with an intact endothelium (> 70% endothelial cover). The sensitivity ($\log_{10} EC_{50}$ values) of the rubbed preparations to angiotensin II, noradrenaline and U46619 was also significantly greater than that of intact tissues.

Furthermore, the responsiveness (maximum tension development) of rubbed tissues to angiotensin II, UK14304 or histamine was significantly greater than that of intact preparations. In the case of noradrenaline, the increased responsiveness was at the borderline of significance at the 5% level whilst for U46619 a significant decrease in responsiveness was observed (Table 1).

Agonist-induced inhibition of noradrenaline-induced contractions

Both histamine and UK14304 induced marked endothelium-dependent inhibition of noradrenaline contractions whereas angiotensin II exhibited only contractile effects (Table 2). Qualitatively similar inhibitory effects were obtained with UK14304 and histamine when tissues were contracted with low concentrations of noradrenaline (20 nM). However, with angiotension II, the threshold concentration, $\log_{10} EC_{50}$ and maximum tension developed were

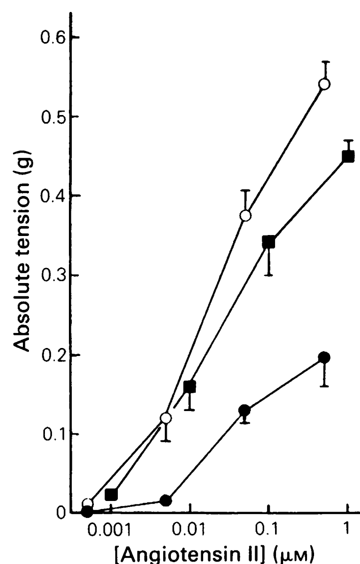

Figure 1 The effects of angiotensin II on tension development by intact (●) and rubbed (○) segments of rat aorta in normal PSS and by intact (■) segments in PSS containing noradrenaline, 20 nM. Ordinate scale: absolute tension development (g). Each point is the mean derived from 6–8 experiments; vertical lines show s.e.mean values.

Table 1 Characteristics of spasmogenic responses to a variety of agonists on rubbed and intact segments of rat aorta

Agonist	n	*Threshold spasmogenic concentration (nM)*		*$\log_{10}$ spasmogenic EC_{50} (nM)*[a]		*Maximum tension development* (E_{max}, g)[b]	
		Rubbed	*Intact*	*Rubbed*	*Intact*	*Rubbed*	*Intact*
Angiotensin II	6	0.5–5	<5	1.25 ± 0.24	1.84 ± 0.13††	0.52 ± 0.04	0.15 ± 0.02†
Histamine	6	<1000	>100000	4.59 ± 1.31	—	0.47 ± 0.04	0.04 ± 0.02†
Noradrenaline	7	<0.5	0.5–2	0.47 ± 0.19	1.35 ± 0.18††	0.78 ± 0.08	0.62 ± 0.08
U46619	8	<0.5	1–5	0.49 ± 0.11	0.95 ± 0.07††	0.74 ± 0.04	0.89 ± 0.06*
UK14304	4	100–1000	1000–10000	2.67 ± 0.35	2.41 ± 0.32	0.52 ± 0.06	0.15 ± 0.04†

— Value could not be calculated due to lack of spasmogenic effect.
† Maximum tension in rubbed tissues significantly greater than that in intact tissues ($P < 0.01$).
* Maximum tension in intact tissues significantly greater than that in rubbed tissues ($P < 0.05$).
†† $-\log_{10} EC_{50}$ values significantly greater in intact than rubbed tissues ($P < 0.05$).
[a] Results presented as mean ± 95% confidence limits.
[b] Results presented as mean ± s.e.mean.

Table 2 Maximal effects of a variety of agonists on spasmogenic responses to noradrenaline (2 μM) on rubbed and intact segments of rat aorta

Agonist	n	Agonist-induced contraction (+) or relaxation (−) as % noradrenaline-induced spasm	
		Rubbed	*Intact*
Acetylcholine (10 μM)	8	0% ± 0.4	− 91% ± 4
Angiotensin II (500 nM)	4	+ 8% ± 5	+ 15% ± 4
Histamine (1 mM)	6	+ 2% ± 0.7	− 51% ± 4
UK14304 (10 μM)	4	− 9% ± 0.5	− 52% ± 13

Data are presented as mean ± s.e.mean.

characteristic of responses to angiotensin II on rubbed tissues in the absence of noradrenaline (Figure 1, Table 1).

Agonist-induced modification of U46619-evoked contractions

On intact preparations contracted with U46619, angiotension II or noradrenaline induced further contractions, acetylcholine and histamine induced concentration-dependent relaxations whilst UK14304 produced a biphasic response, consisting of a small relaxation followed by a contraction (Table 3, Figure 2).

The effects of UK14304 and noradrenaline were further investigated on rubbed and intact preparations in the presence of prazosin (1 μM), a concentration which abolished the contractile effect of noradrenaline (1 μM). When contractions were induced with U46619 on intact preparations, subsequent challenge with UK14304 (0.1–10 μM) induced dose-dependent relaxations which were unaffected by propranolol (1 μM) but were abolished by phentolamine (1 μM) (Figure 3). Similarly, noradrenaline (1–10 μM) induced endothelium-dependent relaxations (Figure 3) which were also abolished by phentolamine (data not shown). The inhibitory effect of acetylcholine was unaffected by the prazosin/propranolol/phentolamine combination (Figure 3). Angiotensin II produced a spasmogenic response on tissues contracted with U46619 in the presence of prazosin (1 μM).

The inhibitory effects of UK14304 and noradrenaline in the presence of prazosin were further investigated in the additional presence of the α_2-adrenoceptor antagonist, idazoxan. Under these conditions, a rightward shift of the UK14304 dose-response curve was observed with no significant change in the maximum inhibitory effect of UK14304 (Figure 4). The pA_2 of idazoxan against UK14304 was 8.51 ± 0.14 ($n = 6$) and the slope of the Schild plot was 1.33 ± 0.17 ($n = 6$). In the presence of idazoxan, the inhibitory concentration-effect curve of noradrenaline was also shifted to the right. However, in the presence of high noradrenaline concentrations, spasmogenic effects were observed which we attribute to an interaction with α_1-adrenoceptors by surmounting prazosin (1 μM) blockade; calculations of the pA_2 value of idazoxan against noradrenaline were impossible.

The effects of pretreatment with idazoxan (0.1 or 1 μM) on spasmogenic responses to UK14304 were studied on matched pairs of rubbed/intact tissues ($n = 4$). Such pretreatment had no significant effect on

Table 3 Maximal effects of a variety of agonists on spasmogenic responses to U46619 (20 nM) on rubbed and intact segments of rat aorta

Agonist	n	Agonist-induced contaction (+) or relaxation (−) as % U46619-induced spasm	
		Rubbed	*Intact*
Acetylcholine (10 μM)	8	+ 2% ± 1.5	− 72% ± 4
Angiotensin II (500 nM)	4	+ 25% ± 4	+ 21% ± 2
Histamine (1 mM)	7	+ 2% ± 0.7	− 46% ± 4
Noradrenaline (1 μM)	4	+ 27% ± 9	+ 42% ± 10
UK14304 (10 μM)	4	+ 19% ± 7	+ 34% ± 7

Data are presented as mean ± s.e.mean.

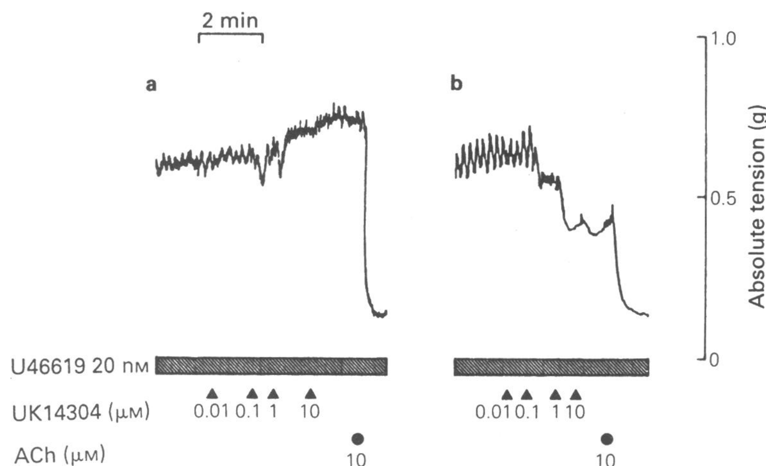

Figure 2 The effects of UK14304 on U46619-induced tension in intact rat aortic strips in (a) the absence of and (b) after 30 min exposure to, prazosin 1 μM. Tissues were exposed to U46619, 20 nM (hatched bar) for 15 min after which they were challenged cumulatively (▲) with UK14304 (0.01–10 μM). Acetylcholine (ACh) 10 μM was used as a functional test of endothelial integrity at the end of the experiment (●). Records from two different strips are shown.

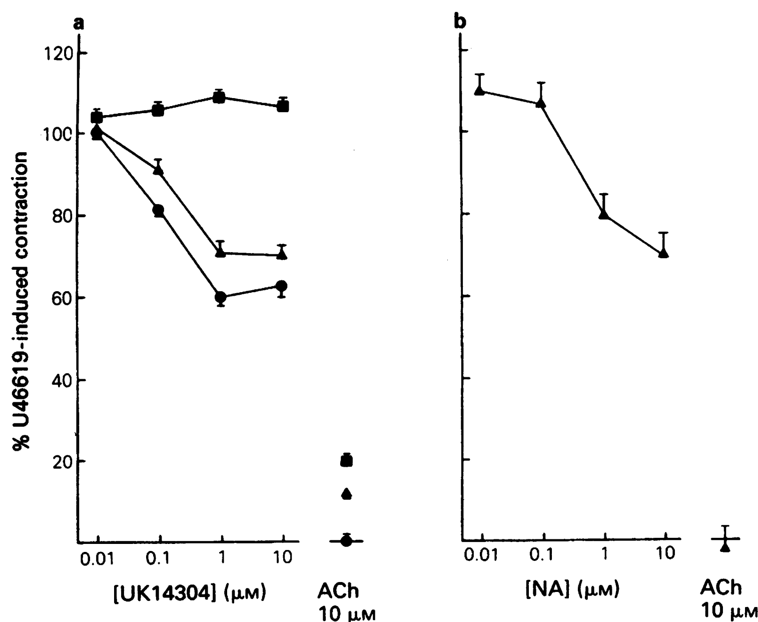

Figure 3 The inhibitory effects of (a) UK14304 and (b) noradrenaline (NA) on intact segments of rat aorta precontracted with U46619 20 nM in the presence of adrenoceptor antagonists. Responses in PSS containing prazosin 1 μM (●); prazosin + propranolol, each 1 μM (▲); prazosin + propranolol + phentolamine, each 1 μM (■). When maximum inhibitory responses to UK14304 or NA had been obtained, tissues were exposed to acetylcholine (ACh) 10 μM in the presence of the antagonists to confirm the presence of the vascular endothelium. Ordinate scale: % U46619-induced contraction. Each point is the mean derived from 4–6 experiments; vertical lines show s.e.mean values.

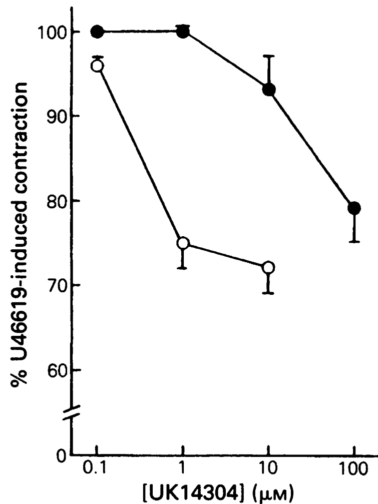

Figure 4 The inhibitory effects of UK14304 on intact segments of rat aorta pre-contracted with U46619, 20 nM in the absence (○) and presence of idazoxan 1 μM (●). Throughout the experiments, the PSS contained prazosin + propranolol (each 1 μM). Data obtained in the presence of idazoxan 0.01 and 0.1 μM have been omitted for clarity. Ordinate scale: % response to U46619, 20 nM. Each point is the mean derived from 6 experiments; vertical lines show s.e.mean values.

the weak contractions produced by UK14304 on intact preparations, or on the more powerful responses mediated by UK14304 on rubbed tissues.

Effects of haemoglobin

Haemoglobin (10 μM) was used to antagonize the effects of EDRF. It had no detectable effect on the basal tone of rubbed or intact tissues or on spasmogenic responses to noradrenaline, UK14304 or U46619 on rubbed preparations. However, responses to these spasmogens on intact preparations were augmented by pre-incubation with haemoglobin and came to equal those obtained on paired, rubbed tissues (Figure 5). The relaxant action of acetylcholine, 10 μM was reversed by exposure to haemoglobin (Figure 6). Furthermore on tissues contracted with U46619 in the presence of prazosin and propranolol (each 1 μM), the inhibitory effects of UK14304 and noradrenaline (each 1–10 μM) were abolished.

Determination of cyclic GMP concentrations

The resting basal concentration of cyclic GMP was significantly lower in the absence than in the presence of the endothelium (0.076 ± 0.02 pmol mg^{-1} protein and 2.26 ± 0.56 pmol mg^{-1} protein, respectively; $P < 0.01$, $n = 4$). Acetylcholine (10 μM) produced a marked increase in cyclic GMP concentration whereas equi-effective mechano-inhibitory concentrations of acetylcholine (30 nM) or UK14304 (10 μM) had no

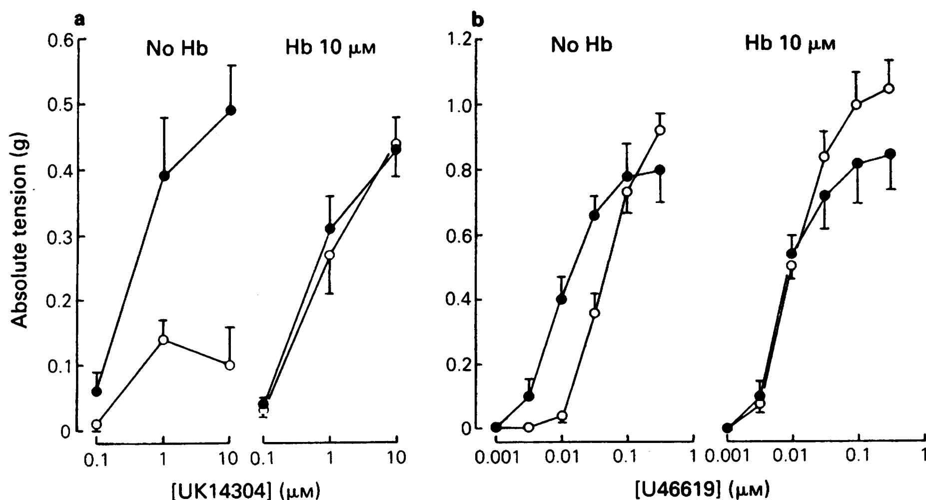

Figure 5 The effects of pre-incubation with haemoglobin (Hb, 10 μM) on spasmogenic responses to (a) UK14304 and (b) U46619 on matched pairs of intact (○) and rubbed (●) segments of rat aorta. Ordinate scale: absolute mechanical responses (g) to either (a) UK14304 or (b) U46619. Each point is the mean derived from 4 experiments; vertical lines show s.e.mean values.

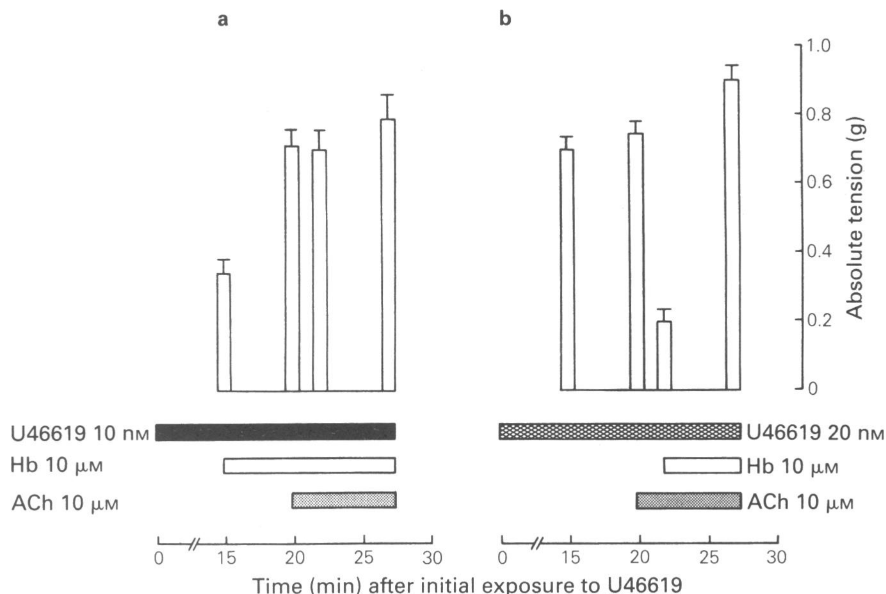

Figure 6 The effects of haemoglobin (Hb) on spasmogenic responses to U46619 and on the relaxant action of acetylcholine (ACh) on matched pairs of (a) test and (b) control intact segments of rat aorta. All tissues were exposed to U46619 (test 10 nM, solid bar; control, 20 nM hatched bar) throughout the experiment. After 15 min exposure to U46619, test tissues were challenged with Hb (10 μM, open bar) followed 5 min later by simultaneous exposure to ACh (10 μM, stippled bar). Control tissues were similarly exposed to ACh followed 2 min later by simultaneous exposure to Hb. Each column represents the mean of 8 experiments; vertical lines show s.e.mean values.

effect (Table 4). Neither of the agents produced significant changes in cyclic GMP levels in rubbed tissues.

Discussion

The objective of the present investigation was to assess the role of the endothelium in modifying the contractile responses of rat aorta to a variety of agonists. When the endothelium was present, spasmogenic threshold concentrations were generally higher than in rubbed preparations and there was a decrease in tissue sensitivity and responsiveness to a given agonist. For angiotensin II, histamine and UK14304, these differences were very marked. Such observations could be explained by assuming that a basal release of EDRF occurs in rat aorta and/or that the spasmogens themselves are capable of liberating EDRF, even at concentrations below the threshold for spasmogenic activity.

Evidence in favour of basal EDRF release is suggested by the difference between the cyclic GMP levels of rubbed and intact tissues (present results; Rapoport & Murad, 1983; Bigaud *et.al.*, 1984) and by the superfusion experiments of Griffith *et al.* (1984). This has led Malta *et al.* (1986) and Martin *et al.* (1986) to propose that basal EDRF release opposes the action of spasmogenic agonists leading to a decrease in tissue sensitivity and responsiveness. Using dibenamine, Martin *et al.* (1986) showed that for phenylephrine at least, the lower the phenylephrine E_{max}, the greater the inhibitory effects of EDRF.

In the present study, angiotensin II was a weak spasmogen on intact aortic segments but induced more powerful contractions in the absence of the endothelium, although its E_{max} was lower than that of noradrenaline. Although Toda (1984) found that angiotensin II produced endothelium-dependent relaxations in canine renal arteries, no evidence was obtained for such an action in the present study. Furthermore, in the presence of a threshold spasmogenic concentration of noradrenaline, the spasmogenic action of angiotensin II was markedly increased. By analogy with the results of Malta *et al.* (1986) and Martin *et al.* (1986), such findings strongly suggest that angiotensin II is a spasmogen with relatively low efficacy. However, in the presence of an additional spasmogen such as noradrenaline, the ability of EDRF to reduce the effectiveness of angiotensin II can be overcome, allowing angiotensin II to produce a full spasm.

Table 4 The effects of acetylcholine and UK14304 on the cyclic GMP concentration in rubbed and intact segments of rat aorta

Pretreatment	*Cyclic GMP concentration* (pmol mg^{-1} protein) *Rubbed*	*Intact*
None	0.076 ± 0.02	2.26 ± 0.58
Acetylcholine (30 nM)	0.065 ± 0.01	2.91 ± 0.41
Acetylcholine (10 μM)	0.098 ± 0.02	16.5 ± 2.6*
UK14304 (10 μM)	0.057 ± 0.01	1.0 ± 0.15
UK14304 (10 μM) in the presence of prazosin (1 μM)	0.102 ± 0.02	2.76 ± 0.36
Prazosin (1 μM)	0.093 ± 0.02	1.04 ± 0.20

Each value is the mean ± s.e.mean of 4 observations determined in duplicate.
* Significantly different from controls, $P < 0.01$.

Histamine was essentially without spasmogenic effect in intact tissues. It became relatively powerful when the endothelium had been removed, but with an E_{max} lower than that of noradrenaline. In contrast to angiotensin II, however, it was possible to demonstrate that histamine was capable of stimulating the release of EDRF confirming the results of other workers (Van de Voorde & Leusen, 1983; Fortes *et al.*, 1983; Rapoport & Murad, 1983; Davies & Williams, 1984; Carrier *et al.*, 1984). It thus seems possible that the differences in the responses of rubbed and intact preparations to histamine result from the reduced effectiveness of histamine on intact preparations due both to basal and to stimulated EDRF release. However, since the actions of histamine on both the smooth muscle and the endothelial cells are mediated by H_1-receptors, the relative contribution of the two components of EDRF cannot be estimated with certainty.

UK14304 is both a partial agonist at α_1-adrenoceptors and an agonist at α_2-adrenoceptors (Cambridge, 1981; Beckeringh *et al.*, 1984; Langer & Hicks, 1984). It behaved as a weak spasmogen when the endothelium was present, but became much more powerful when the endothelium was removed. Its E_{max}, however, remained lower than that of noradrenaline, The spasmogenic action of UK14304 was abolished by prazosin indicating that the effect was mediated by α_1-adrenoceptors (Langer & Hicks, 1984). When intact tissues were contracted with noradrenaline, UK14304 relaxed the spasm, indicating the possibility of stimulated EDRF release. However, when U46619, the stable thromboxane A_2-mimetic (Coleman *et al.*,1981) was used as a spasmogen instead of noradrenaline, UK14304 produced only a short-lived inhibitory effect followed by a small spasm. It thus seems likely that the UK14304-mediated relaxation of a noradrenaline spasm is a reflection of a partial agonist effect at α_1-adrenoceptors.

To test whether the transient inhibitory action of UK14304 against a U46619 spasm was mediated by α_2-adrenoceptors, the effects of UK14304 were studied in the presence of an α_1-adrenoceptor blocking concentration of prazosin. Under these conditions UK14304 produced a concentration-dependent inhibition of U46619-induced contractions on intact tissues alone. This inhibitory effect was not antagonized by propranolol but could be blocked either with phentolamine (an α_1/α_2-adrenoceptor antagonist, Langer & Shepperson, 1982) or with idazoxan (a selective α_2-adrenoceptor antagonist, Doxey *et al.*, 1984; Langer & Hicks, 1984). The pA_2 value of idazoxan against the endothelial-dependent inhibitory action of UK14304 was characteristic of an effect at α_2-adrenoceptors (Doxey *et al.*, 1984).

Furthermore, noradrenaline was capable of producing a similar endothelium-dependent inhibitory response in the presence of prazosin and the inhibitory effects of both noradrenaline and UK14304 were abolished by haemoglobin. The possibility that the inhibitory effects of UK14304 and noradrenaline were produced by the liberation of histamine was excluded by the use of mepyramine, which failed to modify the relaxations produced by either agonist (Taylor & Weston, unpublished observations). These results provide clear evidence for the existence on endothelial cells of α_2-adrenoceptors, stimulation of which results in EDRF release. In this respect therefore, the endothelium of the rat aorta is similar to that of various canine, porcine and human blood vessels (Cocks & Angus, 1983; Calvete *et al.*, 1984; Miller & Vanhoutte, 1985; Angus *et al.*, 1986).

To date, no clear evidence for the presence of α_2-adrenoceptors on the rat vascular endothelium has been presented and several workers have concluded that such receptors do not exist (Dashwood & Jacobs, 1985; Godfraind *et al.*, 1985; Murakami *et al.*, 1985; Martin *et al.*, 1986). However, Godfraind *et al.* (1985)

and Murakami *et al.* (1985) did not perform their experiments in the presence of α_1-adrenoceptor blockade and thus any inhibitory effects of α_2-adrenoceptor stimulation were masked. Martin *et al.* (1986) have observed that in a single experiment in intact rat aorta, clonidine produced no relaxation of a prostaglandin $F_{2\alpha}$-induced spasm in the presence of prazosin. In view of the results obtained in the present study this is a surprising observation but further experimental evidence relating to this apparent anomally is required to allow further comment.

The autoradiographic technique used by Dashwood & Jacobs (1985) is relatively insensitive and this, together with the much greater proportion of α_1-than α_2-adrenoceptor binding sites in rat aorta (Descombes & Stoclet, 1985) must have contributed to the inability of these workers to detect any α_2-adrenoceptors. Furthermore, the selectivity of rauwolscine, used as a marker for α_2-adrenoceptors by Dashwood & Jacobs (1985) has been questioned (Doxey *et al.*, 1984).

Martin *et al.* (1986) were unable to detect any increase in cyclic GMP levels in rat aorta following exposure to α_2-agonists and on this basis they concluded that α_2-adrenoceptor mediated EDRF release was absent. We also failed to detect any such UK14304-induced changes in the presence or absence of prazosin. However, we were also unable to detect any significant changes in cyclic GMP concentrations in the presence of a concentration of acetylcholine which produced a relaxation equivalent to the maximum obtainable with UK14304 (approximately 30%). We suggest therefore that changes in cyclic GMP concentrations associated with relatively small relaxations cannot be satisfactorily detected with present methods. Similar comments have been made by other workers (see, for example, Bowman & Drummond, 1984).

Experiments with the α_2-adrenoceptor antagonist idazoxan were performed to provide an estimate of the extent to which basal and/or α_2-adrenoceptor mediated EDRF release contributed to the poor sensitivity and responsiveness of intact preparations to UK14304. In the presence of idazoxan, no increase in the sensitivity or responsiveness to UK14304 was detected. These results, together with those obtained by Lues & Schümann (1984) and Godfraind *et al.* (1985) using the antagonist rauwolscine and the agonists guanfacine and St 587 suggest that basal rather than stimulated release of EDRF is the main contributor to depression of vasoconstrictor responses in rat aorta.

In rubbed preparations, the noradrenaline E_{max} was greater than that of angiotensin II, histamine or UK14304. However in the presence of the endothelium, the resulting decrease in the noradrenaline E_{max} was less than that of angiotensin II, histamine or UK14304. Martin *et al.* (1986) have shown that when the E_{max} of phenylephrine was lowered using dibenamine, the ability of EDRF to oppose the contractile activity of phenylephrine was markedly increased. The results of the present study using noradrenaline and UK14304 are in agreement with this observation. Furthermore the additional data obtained using angiotensin II and histamine suggest that in general, the lower the agonist efficacy the greater will be the inhibitory effect of the endothelium.

It seems reasonable to conclude that some, at least, of these observations are of physiological and possibly clinical significance. Damage to the endothelium will result in an increased sensitivity and responsiveness to both α_1-adrenoceptor agonists and to angiotensin II. In vascular disease in which the endothelium is damaged, such changes could be relevant to subsequent vascular reactivity. In certain vascular beds the inhibitory role of α_2-adrenoceptors located in the endothelium may also be more important than in rat aorta. Cocks & Angus (1983) and Angus *et al.* (1986) have demonstrated very marked relaxations mediated by α_2-adrenoceptors in canine and porcine arteries.

The effects of sympathomimetics, angiotensin II, histamine and UK14304 on rubbed and intact preparations can be adequately explained by the inhibitory actions of EDRF. However, this explanation does not completely account for the results obtained with U46619. The sensitivity of intact tissues to this thromboxane A_2-mimetic was less than that of rubbed preparations, a difference which was abolished by incubation with haemoglobin. This is consistent with an action resulting from the basal release of EDRF. However, the U46619 E_{max} was paradoxically greater on intact than on rubbed preparations in the presence or absence of haemoglobin. The significance of this observation is being further studied.

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