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Pharmacological reactivity of human epicardial coronary arteries: characterization of relaxation responses to endothelium-derived relaxing factor

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1 Human epicardial coronary artery rings, freshly obtained from cardiac transplant patients, were examined for their responses to endothelium-derived relaxing factor (EDRF)-releasing agents.

2 Functional antagonism profoundly influenced relaxation responses in this tissue. Increasing force with concentrations of U46619 above 3 nM (40% of maximum contraction response) resulted in a reduction of the maximum response to four vasorelaxants which relax vascular smooth muscle via different mechanisms: the EDRF-releasing agents, substance P and bradykinin; the endothelium-independent nitro-vasodilator, sodium nitroprusside (SNP); and the β-adrenoceptor agonist, isoprenaline.

3 Substance P, histamine, bradykinin and the Ca^{2+} ionophores ionomycin and A23187 all caused concentration- and endothelium-dependent relaxation in vessels pre-contracted with the thromboxane A_2-mimetic, U46619 (3 nM) to an active force optimal for relaxation responses. Nifedipine (0.1 μM), added to prevent spontaneous contractions, had no effect on relaxation responses to substance P, bradykinin and histamine.

4 Substance P was the most potent of the EDRF-releasing agents examined and all agents except for bradykinin caused near-maximal relaxation. Bradykinin caused only 46.2% ± 7.3% relaxation. Responses were abolished when the endothelium was removed and, except for histamine, were not significantly affected by indomethacin (3–10 μM, $P > 0.05$). Histamine (0.1–10 μM) caused a concentration-dependent contraction of arterial rings without endothelium.

5 The L-arginine analogues N^G-nitro-L-arginine (L-NOARG, 0.1 mM) and N^G-monomethyl-L-arginine (L-NMMA, 0.1 mM) both caused no further contraction in arteries precontracted with U46619 (3 nM) and were in general, poor inhibitors of responses to EDRF agonists. L-NMMA, but not L-NOARG, caused small but significant decreases in the maximum responses to substance P, bradykinin (18.5 ± 6.9% and 27.6 ± 10.9% relaxation with L-NMMA and L-NOARG, respectively), histamine and A23187 ($P < 0.05$). The analogues had no effect on SNP responses.

6 In conclusion, EDRF release in human isolated coronary artery is only poorly antagonized by the nitric oxide synthase inhibitors L-NOARG and L-NMMA. These results indicate that either the nitric oxide transduction pathway present in human coronary artery is different from that in other tissues or that another factor(s) (e.g. endothelium-derived hyperpolarizing factor) is also released in response to EDRF-releasing agents and augments the relaxation to nitric oxide.

Keywords: Human coronary artery; endothelium-derived relaxing factor; nitric oxide; substance P; bradykinin; L-arginine analogues; Ca^{2+} ionophores; isoprenaline; functional antagonism

Introduction

The importance of endothelium-derived relaxing factor (EDRF) in the regulation of vascular reactivity has been of interest since Furchgott & Zawadzki (1980) first described the role of the endothelium in mediating relaxation responses to some vasodilator agents.

Investigations in human coronary artery *in vivo* for evaluation of endothelial dysfunction in association with atherosclerosis have revealed that substance P (Crossman *et al.*, 1989; Kushwaha *et al.*, 1991; Yamamoto *et al.*, 1992) and to some extent, acetylcholine (Ludmer *et al.*, 1986; Yasue *et al.*, 1990) cause potent vasodilatation of human epicardial coronary arteries. Acetylcholine has been shown to be a sensitive marker of endothelial dysfunction with early atheroma (Vita *et al.*, 1990; Zeiher *et al.*, 1991), whilst substance P is a powerful vasodilator agent which can increase arterial diameter by up to 30% (Crossman *et al.*, 1989).

Toda (1987) demonstrated a relaxation response to substance P in human coronary artery helical strips *in vitro*, and release of EDRF by histamine in the same tissue. Bradykinin, the calcium ionophore A23187 and, occasionally, acetylcholine, have also been shown to relax human coronary artery *in vitro* (Förstermann *et al.*, 1988).

Palmer *et al.* (1987) showed that the response of EDRF was identical to that seen to nitric oxide (NO). The source for EDRF (NO) is the guanidino nitrogen atom of arginine (Palmer *et al.*, 1988). Since these discoveries, various L-arginine analogues have been used experimentally in a number of tissues to block activity in the enzyme which converts L-arginine to NO, nitric oxide synthase (see Moncada *et al.*, 1991): N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-L-arginine (L-NOARG), N^G-methyl-L-arginine (L-NMA) and N^G-monomethyl-L-arginine (L-NMMA), amongst others (see Moncada *et al.*, 1991 for an overview). In human coronary arteries, however, only the effect of L-NMMA on the EDRF response to substance P has been examined, with the L-arginine analogue reported to decrease the maximum relaxation response from 89.1 ± 8.5% to 34.0 ± 10.5% of the glyceryl trinitrate response (Chester *et al.*, 1990a,b).

The first part of this study was designed to establish optimal conditions in human isolated epicardial coronary

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artery in order to examine the effects of endothelium-dependent vasorelaxing agents. Some of these conditions were described in the preceding paper, where we found that nifedipine abolished spontaneous phasic contractile activity, but tonic contractions to the thromboxane A_2-mimetic U46619 were unaffected by the calcium channel antagonist (Stork & Cocks, 1994). The importance of any functional (physiological) antagonism of relaxation responses via increasing levels of U46619-induced active force, and the effect of nifedipine on release of EDRF were determined. Once optimal conditions for EDRF-mediated relaxations were established, the response to the EDRF (NO)-releasing agents substance P, bradykinin, histamine, ionomycin and A23187 (the latter two being Ca^{2+} ionophores) were characterized. The effect of the L-arginine analogues L-NOARG and L-NMMA on these responses, as well as on basal or U46619-induced active force, were also investigated.

A preliminary account of this study was presented at the 26th annual meeting of the Australian Society of Clinical and Experimental Pharmacologists and Toxicologists (Stork & Cocks, 1992).

Methods

Human coronary arteries

Epicardial coronary arteries were obtained from the explanted hearts of 31 patients (21 male, 10 female) involved in the Alfred Hospital Heart & Lung Transplant Service who signed consent forms prior to surgery. Patients were diagnosed as having dilated cardiomyopathy ($n = 17$), ischaemic heart disease ($n = 9$), congenital heart disease ($n = 1$) or mitral valve disease ($n = 1$). Three donor hearts from primary pulmonary hypertensive patients, unsuitable for transplantation, were also used. Average age was 46.5 years with a median age of 52 years (range 19–66).

Epicardial coronary arteries were dissected from hearts within 20 min of excision and taken to the laboratory in cold Krebs solution (4°C, see below for composition). Arteries were then further dissected free of any surrounding myocardium and fatty tissue and cut into 3 mm long ring segments. Only rings of arteries without gross macroscopic evidence of atherosclerosis were included. In total, 373 rings from nine circumflex, 15 left anterior descending and 13 right coronary arteries, six primary circumflex, six primary left anterior descending and eight primary right coronary artery branches were used. Endothelium was left intact in most cases and was removed from a few rings by mechanical rubbing with a tapered wooden stick to check for endothelium-dependency of the responses.

The vessel segments were set up in 30 ml organ baths containing Krebs solution [consisting of (in mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2 and glucose 11] at 37°C and aerated with a 95% O_2/5% CO_2 gas mixture. Two L-shaped wire hooks (355 μm in diameter) were passed through the vessel lumen. One wire was attached to a stationary support leg attached to a manually-driven micrometer, while the other wire was attached to an isometric force transducer (Grass Instruments, model FT03C) to measure force development. Force was recorded on either single (Rikadenki) or dual (W&W Scientific Instruments) flat bed pen recorders.

The arterial rings were allowed to equilibrate for 60 min before being subjected to two passive stretches to 5 g force at 30 min intervals (resulting in an optimal passive tension). Nifedipine (0.1 μM) was then added to remove and prevent phasic contractile activity (see Stork & Cocks, 1994). Where the effect of the L-arginine analogues on the agonists were examined, the analogues were added and allowed to equilibrate for 30 min. The vessels were then precontracted with the thromboxane A_2-mimetic, U46619 (1–30 nM) and cumulative concentration-response curves to various vasorelaxant drugs were constructed at half-log unit intervals once a steady level of active force was obtained. In some experiments, nifedipine was not used and the vessels were contracted with 1 nM U46619 due to the increased sensitivity of the phasically active tissue (Stork & Cocks, 1994). Only one protocol and curve per ring per patient was performed.

Drugs

The following drugs were used: histamine dihydrochloride, ionomycin free acid and A23187 free acid (Calbiochem); substance P acetate, bradykinin acetate, (−)-isoprenaline bitartrate and N^G-nitro-L-arginine (L-NOARG) (Sigma); N^G-monomethyl-L-arginine (L-NMMA, International Drug Technology, Melbourne, Australia); sodium nitroprusside (SNP, Roche); U46619 [1,5,5-hydroxy-11α,9α(epoxymethano) prosta-E 2,13-dienoic acid] (Upjohn); endothelin-1 (Peninsula Labs, USA) and (−)-nifedipine (Bayer).

Nifedipine (10 mM) was made up in 100% ethanol on the day of the experiment. Stock solutions of L-NOARG (0.1 M) and the Ca^{2+} ionophores, ionomycin and A23197 (both 1 mM), were made up in 1 M $NaHCO_3$ and 100% ethanol, respectively. All other drugs were from stock solutions made up in distilled water and all dilutions were in distilled water.

Statistics and data analysis

Individual relaxation responses to the agonists were converted to percentages of the maximum possible relaxation, as assessed by the level of active force developed to U46619. The constrictor effects of the L-arginine analogues, L-NOARG and L-NMMA, were measured in g force, due to wide variation when converted to percentage values of the initial contraction to U46619.

The individual concentration-response curves were then computer-fitted to the sigmoidal logistic equation

$$Y = P_1 + P_2/[1 + e^{P_3(\log X - P_4)}],$$

where X = agonist concentration, P_1 = lower plateau response, P_2 = range between the lower and the maximal plateau of the concentration response curve, P_3 = a negative curvature index indicating the slope independently of the range and P_4 = log dose required to produce a half-maximal response (EC_{50}) (Elghozi & Head, 1990). These calculations were used to determine pEC_{50} (− log M) values. All values are presented as the mean ± standard error of the mean (s.e. mean) for the given number of experiments (n).

Student's unpaired t test was used to compare two independent groups of data and one-way analysis of variance (ANOVA) with Scheffe's test was used to make comparisons between multiple individual groups (Wallenstein *et al.*, 1980). The level of significance was set at $P \leqslant 0.05$.

Results

Optimal conditions

Concentration-relaxation response curves to substance P ($n = 5$), bradykinin ($n = 5$), and histamine ($n = 2$, data not shown) were determined in vessels which held a stable contraction to U46619 (1 nM) in the absence of nifedipine (Figure 1). In the absence of nifedipine, substance P caused 89.7 ± 4.8% relaxation ($pEC_{50} = 10.02 \pm 0.13$) and bradykinin caused 47.2 ± 12.6% relaxation ($pEC_{50} = 8.48 \pm 0.13$). The presence of nifedipine (0.1 μM) abolished phasic contractions and did not affect the relaxation responses to these agents ($P > 0.05$, see below for data in the presence of nifedipine). Consequently, nifedipine (0.1 μM) was routinely used in all following experiments to abolish phasic activity and ensure a steady level of active force to U46619.

The magnitude of relaxation responses to substance P, bradykinin, sodium nitroprusside (SNP) and isoprenaline

depended on the amplitude of contractions to U46619 (Figure 2). At 1, 3, 10 and 30 nM, U46619 caused contractions of approximately 15%, 40%, 70% and 85%, respectively, of the constrictor-maximum in this tissue (Stork & Cocks, unpublished data). In arterial rings contracted with 1 and 3 nM U46619, the relaxation curves to all relaxant agents were superimposable, with similar pEC_{50} values and maximum responses [pEC_{50} values in 3 nM U46619-contracted tissues = 9.93 ± 0.15 (substance P); 8.45 ± 0.17 (bradykinin); 7.85 ± 0.12 (SNP) and 6.40 ± 0.21 (isoprenaline); $n = 5$ for each agonist]. At 10 nM U46619, maximum responses were decreased, although this just failed to reach statistical significance ($P > 0.05$, ANOVA). At 30 nM U46619 there was a significant decrease in the maximum response for all four relaxant agents when compared with the responses in rings contracted with 1 and 3 nM U46619 ($P < 0.05$) and a significant decrease in the pEC_{50} value for SNP with similar comparisons ($pEC_{50} = 6.40 \pm 0.31$, $P < 0.05$). Relaxation responses to bradykinin were abolished at 30 nM U46619 (Figure 2b). Consequently, all relaxation experiments were performed in vessels contracted with 3 nM U46619.

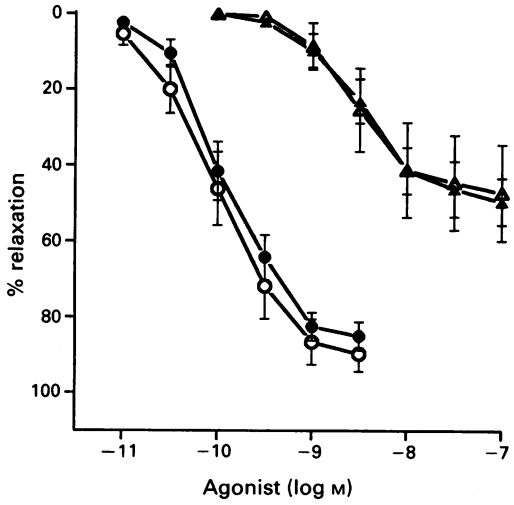

Figure 1 Absence of effect of nifedipine (0.1 μM) on the relaxation response to substance P (○, ●) and bradykinin (△, ▲) in human coronary artery. Concentration-response curves were performed in the absence (○, △, $n = 5$ for both agonists) and presence of nifedipine (●, ▲, $n = 11$ and 10 for substance P and bradykinin, respectively).

EDRF

The EDRF-releasing agents substance P, bradykinin, histamine, ionomycin and A23187 all caused concentration- and endothelium-dependent relaxation responses in U46619-precontracted human coronary artery rings.

Substance P was the most potent of the endothelium-dependent relaxants examined. Threshold responses occurred at 10–30 pM with an estimated pEC_{50} value of 9.94 ± 0.10 ($n = 11$, Table 1). The maximum relaxation response to substance P was 85.2% ± 3.7% at 3 nM ($n = 11$, Table 1, Figure 3a). Addition of 10 μM SNP caused complete relaxation of the tissue (data not shown).

Bradykinin was more potent than histamine (Table 1) but histamine was more effective, causing a maximum relaxation

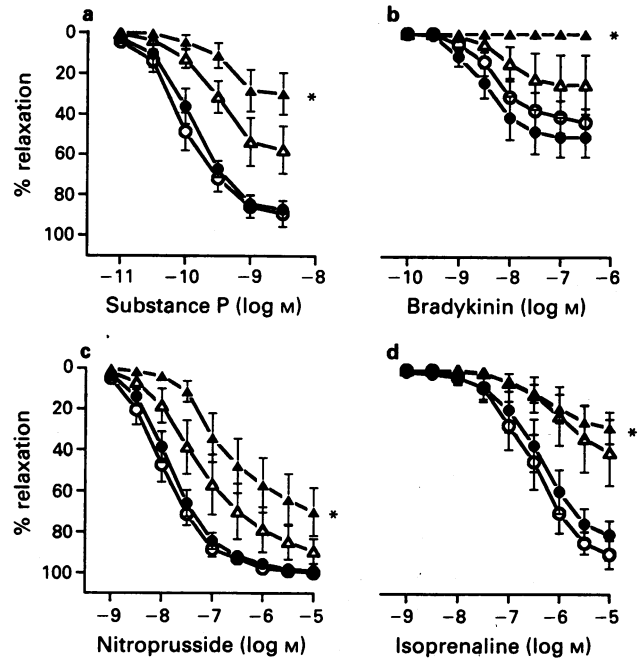

Figure 2 Effect of functional antagonism on relaxation responses in human coronary artery. Arterial rings were contracted with (○) 1 nM, (●) 3 nM, (△) 10 nM and (△) 30 nM U46619. Mean cumulative concentration-relaxation curves are shown for (a) substance P, (b) bradykinin, (c) sodium nitroprusside and (d) isoprenaline. Values are the mean ± s.e.mean of 4–6 experiments. *Indicates $P < 0.05$ (ANOVA) for maximum response in 30 nM- vs 1 nM- and 3 nM-contracted rings.

Table 1 Estimated pEC_{50} values and relaxation maxima (% R) for EDRF agonists in human coronary arteries in the absence and presence of N^G-nitro-L-arginine (L-NOARG, 0.1 mM) and N^G-monomethyl-L-arginine (L-NMMA, 0.1 mM)

		Control	*L-NOARG*	*L-NMMA*
Substance P	(pEC_{50})	9.94 ± 0.10 (11)	9.63 ± 0.08 (9)	9.69 ± 0.12 (7)
	(%R)	85.2 ± 3.7% (11)	69.5 ± 7.3% (10)	58.7 ± 7.2% (7)†
Bradykinin	(pEC_{50})	8.42 ± 0.10 (9)	8.26 ± 0.21 (6)	8.01 ± 0.25 (5)
	(%R)	46.2 ± 7.3% (7)	27.6 ± 10.9% (7)	18.5 ± 6.9% (7)†
Histamine	(pEC_{50})	7.20 ± 0.12 (7)	6.79 ± 0.14 (7)	7.11 ± 0.10 (7)
	(%R)	81.5 ± 2.6% (7)	72.8 ± 7.2% (5)	69.6 ± 11.7% (5)†
Histamine + indomethacin	(pEC_{50})	6.48 ± 0.14 (5)	5.98 ± 0.20 (4)	6.47 ± 0.32 (4)
	(%R)	75.3 ± 8.6% (5)	63.6 ± 3.1% (4)	70.3 ± 8.3% (4)
Ionomycin	(pEC_{50})	7.76 ± 0.11 (7)	7.28 ± 0.15 (6)	7.36 ± 0.18 (7)
	(%R)	87.5 ± 8.6% (7)	52.8 ± 12.3% (7)*	80.2 ± 5.4% (7)
A23187	(pEC_{50})	7.12 ± 0.15	6.63 ± 0.17 (7)	6.71 ± 0.21 (7)
	(%R)	92.8 ± 2.0% (8)	75.2 ± 5.3% (7)	68.4 ± 7.3% (7)†
SNP	(pEC_{50})	7.90 ± 0.16 (6)	7.87 ± 0.20 (4)	8.03 ± 0.27 (4)
	(%R)	100.0 ± 0.0% (6)	100.0 ± 0.0% (4)	100.0 ± 0.0% (4)

Values are mean ± s.e.mean for (n) experiments. *Indicates values significantly different from all others; †indicates values significantly different from control ($P < 0.05$, ANOVA).

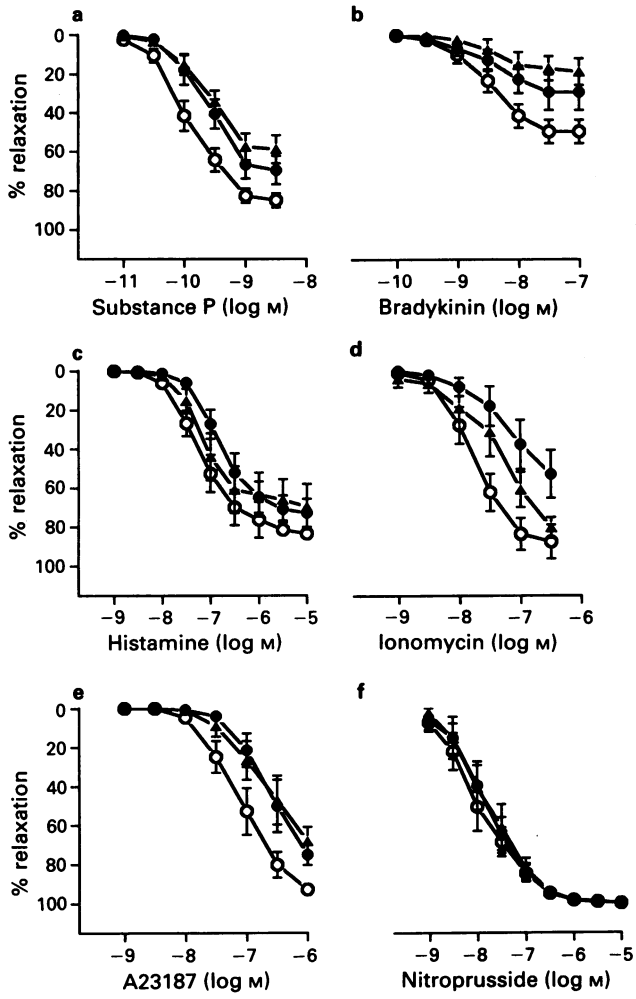

Figure 3 Mean cumulative concentration-relaxation curves for EDRF-releasing agents in human coronary artery. Arterial rings were: (○) control; (●) N^G-nitro-L-arginine-treated (0.1 mM) and (△) N^G-monomethyl-L-arginine-treated (0.1 mM). Agents examined were (a) substance P, (b) bradykinin, (c) histamine, (d) A23187, (e) ionomycin and (f) the endothelium-independent dilator, sodium nitroprusside. Mean pEC_{50} and maximum relaxation values are given in Table 1.

equal to that for substance P (Figure 3c). Bradykinin, on the other hand, caused only 46.2% ± 7.3% relaxation at 0.1 μM (Figure 3b, $n = 10$). Addition of 1 nM substance P after the bradykinin concentration-response curve caused further relaxation, resulting in a total endothelium-dependent response of 84.1% ± 4.6% relaxation ($n = 6$, data not shown). Threshold responses were obtained at concentrations of 0.3–1 nM for bradykinin and 3–10 nM for histamine.

Pretreatment of arterial rings with indomethacin (3–10 μM) did not significantly alter the responses to substance P or bradykinin ($n = 5$ and 4, respectively, data not shown) but the pEC_{50} value for histamine was significantly increased from 7.20 ± 0.12 to 6.48 ± 0.14 ($n = 5$, Table 1, $P < 0.05$, t test). The maximum response to histamine, however, was not affected by indomethacin (Table 1).

The Ca^{2+} ionophores, ionomycin and A23187, also caused near-maximal relaxation of U46619-precontracted arterial rings (Figure 3d,e). Ionomycin was more potent than A23187, with threshold responses at 3 nM, compared with 10 nM for A23187, and a greater pEC_{50} value (Table 1).

Possible release of EDRF by endothelin-1 was also examined. No relaxation responses, however, were seen over the concentration range 0.1–100 pM and at 300 pM, further contraction occurred (data not shown).

Relaxation responses to substance P, bradykinin and histamine were abolished by removal of the endothelium ($n = 2$ for each drug; data not shown). Histamine, in concentrations above 0.1 μM, caused further contraction of U46619 precontracted endothelium-denuded rings (data not shown).

SNP (10 μM) caused maximal (100%) relaxation of all arterial rings. Threshold responses were between 1–3 nM with a pEC_{50} value of 7.90 ± 0.15 nM ($n = 6$, Table 1, Figure 3f).

L-Arginine analogues

The L-arginine analogues L-NOARG and L-NMMA (both 0.1 mM) caused no contraction responses when added to uncontracted rings. Similarly, cumulative addition of either analogues (1–100 μM) following establishment of stable contractions to U46619 (3 nM) caused no significant further increases in active force compared with time controls (from 5.0 ± 1.2 g to 5.1 ± 1.2 g with 100 μM L-NOARG and from 6.8 ± 1.5 g to 7.4 ± 1.6 g with 100 μM L-NMMA; $P > 0.05$ for both, t test). In further experiments, haemoglobin (1 μM, $n = 3$) and methylene blue (10 μM, $n = 3$) also had little or no further contractile effect on the contraction to U46619.

In general, both L-NOARG and L-NMMA were poor inhibitors of relaxation responses to all EDRF-releasing agents (Table 1, Figure 3a–e). L-NMMA caused a small but significant ($P < 0.05$, ANOVA) inhibition of the maximum responses to all agents except ionomycin, which was the only agent against which L-NOARG caused a small but significant depression of the maximum response (Table 1).

Whilst L-NOARG and L-NMMA appeared to cause rightward shifts of the concentration-response curves of some of the EDRF-releasing agents, on statistical analysis, the changes in the pEC_{50} values were not significant ($P > 0.05$, ANOVA, (Table 1). The concentration-relaxation curve for SNP was unaffected by either analogue (Figure 3f).

Both the degree and pattern of inhibition by the L-arginine analogues on relaxation responses to susbtance P and bradykinin were unaffected by pretreatment with indomethacin (3–10 μM; $P > 0.05$, t test). Even with histamine responses in the presence of indomethacin, L-NOARG and L-NMMA still had no significant further inhibitory effects on either pEC_{50} values or the maximum responses (Table 1, $P > 0.05$, ANOVA, $n = 4$ for both analogues).

Discussion

This study has defined the optimal conditions for examining relaxation responses in human coronary artery *in vitro*. The two major determinants for these conditions were removal of phasic contractile activity and avoidance of functional antagonism by the agent used to precontract the tissues. Phasic contractile activity, particularly that which occurs in response to contractile agents like U46619, is characteristic of this tissue (Cocks *et al.*, 1993; Stork & Cocks, 1994). We have shown that nifedipine not only abolishes this activity, but also, importantly, has no effect on tonic contractions to U46619 (Stork & Cocks, 1994). Thus nifedipine allowed us to determine full concentration-dependent relaxation-response curves to various relaxing agents and evaluate the importance of the level of precontraction on these responses. Nifedipine had no inhibitory effect on relaxation responses to any EDRF-releasing agents used in this tissue, in agreement with previous studies in dog coronary artery (Angus & Cocks, 1989) and rat aorta (Schoeffter & Miller, 1986).

Functional antagonism was found to be an important factor in determining the maximum response to endothelium-dependent and independent vasorelaxants. There is little uniformity in the literature on the constrictors used, their concentrations and the level of active force present when determining relaxation responses in human isolated coronary artery (compare Förstermann *et al.*, 1986; 1988;

Chester *et al.*, 1990a,b). We found that above approximately 40% of the U46619-maximum in this tissue, functional antagonism became an increasingly influential factor. The pattern of relaxant curve shifts in response to increasing levels of active force appeared to fit the type IIb model of functional antagonism proposed by Mackay (1981): i.e. depression of the maximum response, but no shift in the location of the curve.

With the optimal conditions for measuring relaxation responses in the human coronary artery defined, we were in a position to clarify possible roles for NO in relaxations to various EDRF-releasing agents.

Substance P was the most potent EDRF-releasing agent examined in this study. The results obtained for this neuropeptide correlate well with those of Chester *et al.* (1990a) in similar preparations, although in our study, substance P was more potent (by approximately a half- to one log unit). Indeed, substance P appears to be the most potent of all dilators in human coronary artery, including the related neuropeptide, calcitonin gene-related peptide (CGRP; McEwan *et al.*, 1986; Franco-Cereceda & Lundberg, 1987) and has been found not to be affected by cyclosporin treatment (O'Neil *et al.*, 1991a,b).

Histamine, although less potent, was as effective in relaxing human coronary artery as substance P, as indicated by similar maximum responses. Toda (1987) suggested that relaxation to histamine was mediated by both H_1 receptors on the endothelium and H_2 receptors in the smooth muscle, but that the response was complicated by contraction via H_1 receptors on the muscle at high concentrations of histamine (0.1 μM and above). Consequently, biphasic curves have often been reported (Toda, 1987; Toda & Okamura, 1989). In this study, however, no contraction responses were observed, even at high concentrations of histamine (1–10 μM), except in the absence of endothelium, indicating that EDRF release (via endothelial H_1 receptors) and stimulation of the (relaxing) H_2 receptors was overriding the direct constrictor effect on the smooth muscle.

Bradykinin, whilst relaxing the arterial rings, caused less than 50% relaxation. This result confirms previous work by Förstermann *et al.* (1988) and Chester *et al.* (1990b) but contradicts the findings of Toda & Okamura (1989), who reported near-maximal relaxation responses to bradykinin. The ability of substance P to cause further relaxation after the maximum response to bradykinin was obtained, indicated that the decreased response with bradykinin was probably not due to factors influencing the release and action of EDRF but that bradykinin is less effective at releasing EDRF in this tissue than the other EDRF agonists (see Martin *et al.*, 1992).

The L-arginine analogues L-NOARG and L-NMMA surprisingly had no effect on either uncontracted or U46619-contracted vessels. Similarly, haemoglobin and methylene blue (two EDRF inhibitors with different mechanisms of action; see Angus & Cocks, 1989) also had little or no contractile effect. This indicates that in the human coronary artery, basal or tonic release of EDRF is poor, at least under *in vitro* conditions. The use of the L-arginine analogues to determine basal release of EDRF, however, has been questioned (Cocks & Angus, 1991).

More surprisingly, L-NOARG and L-NMMA had little antagonistic effects on the relaxation responses to a variety of EDRF-releasing agents, including bradykinin, which had the lowest relative efficacy. Previous findings in other vascular tissues show L-NOARG and L-NMMA to be effective blockers of EDRF release, with L-NOARG or its metabolic precursor L-NAME more potent and effective than L-NMMA (Mülsch & Busse, 1990; Rees *et al.*, 1990). However, this was not demonstrated in our study and in fact, the trend was reversed, with L-NMMA being more effective than L-NOARG in decreasing the maximum response to most of the relaxants.

Martin *et al.* (1992) pointed out that there are a number of factors which influence the release of EDRF, its blockade by the L-arginine analogues and the final, observed effect. Cell entry and enzyme affinity (which could be affected by isoform differences) obviously characterize the analogues and influence their effectiveness. Levels of intracellular L-arginine can also influence the effectiveness of the L-arginine analogues at inhibiting the enzyme, as they compete for the same site. Agonist-receptor coupling and enzyme activation characteristics of the EDRF-releasing agent used also affect the observed response, as do target cell activation characteristics (see Martin *et al.*, 1992).

The model of Martin *et al.* (1992) for EDRF release and action, together with L-arginine analogue interaction and pharmacokinetics, predicts parallel displacement of high-efficacy agonists and combination of curve-shifts with maxima depression for low-efficacy agonists. In our study, however, only depressed maxima were observed for all EDRF-releasing agonists examined, probably indicating low-efficacy agonists. This is supported by a small displacement to the right and a significant reduction in the maximum of the relaxation curves to substance P and bradykinin with functional antagonism. Correspondingly, the L-arginine analogues could be expected to be effective blockers of EDRF release in this tissue. Nevertheless, the response to bradykinin, the least-effective agent in this tissue, could not be abolished in the presence of L-NOARG and L-NMMA, which, if bradykinin-induced relaxation were mediated by nitric oxide, suggests ineffective blockade of the enzyme. Further, decreasing the pEC_{50} for histamine with indomethacin also failed to increase the ability of the L-arginine analogues to shift the histamine curve or depress the maximum response. These results support the idea that human coronary artery may either possess a different isoform of nitric oxide synthase or a different transduction pathway, or other factors apart from nitric oxide may contribute to the endothelium-dependent relaxation response.

The involvement of prostacyclin in the relaxation response to the EDRF-releasing agents used here was found to be minor. Only responses to histamine, a known releaser of prostacyclin, were affected by cyclo-oxygenase inhibition with indomethacin, confirming previous work which found no evidence for a role for prostacyclin in human coronary artery *in vitro* (Chester *et al.*, 1990a; Toda, 1987). This is despite using the constrictor agent on which prostacyclin and EDRF are at their most effective (U46619; Noll *et al.*, 1991). The possibility that another factor, endothelium-derived hyperpolarizing factor (EDHF; Taylor & Weston, 1988) contributes to the relaxation response cannot be ruled out and its role in human coronary artery requires further research.

The physiological role of EDRF remains uncertain, although according to Moncada *et al.* (1991) '... the cardiovascular system is in a constant state of active vasodilatation dependent on the generation of [nitric oxide].' Our results indicate that in human coronary artery, at least *in vitro*, EDRF is released by a number of vasodilator agents and is relatively unaffected by the L-arginine analogues L-NOARG and L-NMMA. If nitric oxide is the EDRF released in response to these agonists, then the possibility of a different transduction pathway in this tissue may account for the poor inhibition of the responses by the L-arginine analogues.

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