



# Physiological antagonism of endothelin-1 in human conductance and resistance coronary artery

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**1** The ability of four endogenous vasodilators, nitric oxide (NO; 0.01–30  $\mu$ M), atrial (ANP), brain (BNP) and C-type (CNP) natriuretic peptide (0.1–300 nM), to reverse endothelin-1 (ET-1; 10 nM) constrictions in human resistance and conductance coronary arteries (CA) *in vitro* was investigated.

**2** ET-1 (0.1–300 nM) constricted resistance CA more potently than conductance CA ( $P < 0.05$ ; EC<sub>50</sub> values 2.98 nM (95% CI: 1.49–5.95 nM and 8.58 (4.72–15.6 nM) respectively)).

**3** The NO-donor diethylamine NONOate fully reversed the ET-1 constriction in conductance CA (E<sub>MAX</sub> 127  $\pm$  9.16%), however only partial reversal was observed in resistance CA (E<sub>MAX</sub> 78.8  $\pm$  8.13;  $P < 0.05$ ). The soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (100  $\mu$ M) reduced the maximum response to diethylamine NONOate to 76.9  $\pm$  14.4% in conductance CA ( $P < 0.05$ ), but had no effect on resistance CA (E<sub>MAX</sub> 77.2  $\pm$  18.4%).

**4** There was no difference between responses to ANP in conductance and resistance CA (EC<sub>50</sub> values 4.25 nM (0.84–21.4 nM) and 18.4 nM (2.92–116 nM), E<sub>MAX</sub> 53.1  $\pm$  14.7% and 48.6  $\pm$  11.8% respectively).

**5** BNP was a more potent vasodilator of conductance than resistance CA. In conductance CA the mean EC<sub>50</sub> value was 2.4 nM (0.74–7.75 nM), E<sub>MAX</sub> 54.5  $\pm$  14.9%. Concentration-response curves to BNP were incomplete in resistance CA.

**6** Concentration-response curves to CNP were incomplete in both conductance and resistance CA.

**7** The greater potency of ET-1 in resistance vessels may exacerbate the effects of increased circulating levels of the peptide in disease. Only NO could fully reverse ET-1 mediated constrictions in conductance CA, and none of the dilators tested could completely counteract constrictions in resistance CA.

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**Keywords:** Human coronary artery; endothelin-1; atrial natriuretic peptide; brain natriuretic peptide; C-type natriuretic peptide; nitric oxide

**Abbreviations:** ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CA, coronary artery; cGMP, guanosine 3'/5'-cyclic monophosphate; CNP, C-type natriuretic peptide; DEA, diethylamine; DEA/NO, diethylamine NONOate; ET-1, endothelin-1; NO, nitric oxide; NOS, nitric oxide synthase; ODO, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; sGC, soluble guanylate cyclase; SNAP, S-nitroso-N-acetyl penicillamine

## Introduction

Endothelin-1 (ET-1) is an important constrictor of human blood vessels, with an unusually long duration of action compared to other endogenous vasoactive compounds (Yanagisawa *et al.*, 1988; Davenport *et al.*, 1989; Franco Cereceda, 1989). It is synthesized and released from the endothelium throughout the human vasculature and to date, no vessel has been reported unresponsive to the peptide. ET-1 is unique amongst constrictor peptides in being released both *via* a regulated pathway in response to external stimuli, as well as being continuously released from a constitutive pathway (Russell *et al.*, 1998). Infusion of the antagonist TAK-44 in normotensive human volunteers results in systemic vasodilatation, indicating a role for ET in the maintenance of basal vascular tone (Haynes *et al.*, 1996).

In pathophysiological conditions, increased levels of the ET peptide have been demonstrated both within athero-

sclerotic vessels (Bacon *et al.*, 1996) and in the plasma of patients with cardiovascular disease (Lerman *et al.*, 1991; Miyauchi *et al.*, 1989). In addition, ischaemic myocytes have been reported to secrete more ET than controls (Wang *et al.*, 1995). Large epicardial coronary arteries (CA) are particularly prone to atherosclerosis, and small resistance CA, although not susceptible to plaque formation, are exposed to ischaemic conditions, as they are downstream of the occluded vessels, and therefore increased levels of ET-1. In order to restore perfusion of the myocardium it is important to understand what substances are capable of reversing the constrictor effects of ET-1 both in conductance and resistance human CA.

As with ET-1, the vasodilator nitric oxide (NO) is continuously released from the endothelium and contributes to the regulation of vascular tone (Palmer *et al.*, 1987; Vallance *et al.*, 1989). Focally, NO levels are increased in atherosclerotic plaques owing to the induction of the enzyme nitric oxide synthase (NOS; Lafond Walker *et al.*, 1997),

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however the overall basal release of NO from the endothelium is decreased in atherosclerotic CA (Chester *et al.*, 1990). Hence the importance of other dilators directly acting on the vascular smooth muscle layer, independent of endothelium-derived NO.

The natriuretic peptides are a group of molecules originally identified as stimulators of sodium and water secretion in the kidney, and have also been shown to have vasodilator function (for review see: Levin *et al.*, 1998). All members contain a conserved 17 amino acid ring structure formed by a disulphide bridge. Atrial natriuretic peptide (ANP) is a 28 amino acid peptide and acts as a circulating hormone (Kangawa & Matsuo, 1984). It is secreted by atrial myocytes in response to stimuli such as stretch, ET and low NO levels. Secretion increases in cardiovascular disease and ventricular myocytes also begin to produce ANP (Saito *et al.*, 1989). Brain natriuretic peptide (BNP) comprises 32 amino acids and is also a circulating hormone. The main site of synthesis is the ventricle, with very little BNP produced by atrial myocytes. Plasma levels of BNP rise in cardiovascular disease and are particularly closely correlated to the severity of cardiomyopathy (Troughton *et al.*, 2000). The 22 amino acid residue C-type natriuretic peptide (CNP) is not secreted by myocytes but is synthesized in the endothelium (Stingo *et al.*, 1992). It is thought to act in a paracrine manner on the vascular smooth muscle layer. Low levels of the peptide are detectable in plasma, although plasma CNP is not elevated in cardiovascular disease (Wei *et al.*, 1993).

Given the potency and long duration of ET-1-mediated constrictions in the human cardiovascular system, we aimed to investigate the extent to which four endogenous vasodilators, NO, ANP, BNP and CNP, could reverse the effects of ET-1 in human endothelium-denuded resistance and conductance CA *in vitro*. Part of this study has previously been reported to the British Pharmacological Society (Wiley & Davenport, 2000).

## Methods

### Tissue collection

CA were obtained (with local ethical approval) from the explanted hearts of 29 patients, mean age 46 years (range 12–60 years; 12 female, 17 male) undergoing transplant operations. Tissue was also collected from four hearts not required for further transplantation, mean age 37 years (range 17–61 years; 3 female, 1 male).

### In vitro pharmacology

**Resistance vessels** Branch CA were isolated from the apex of the hearts with the aid of a dissecting microscope. Surrounding fat and connective tissue was removed and 1–2 mm segments were mounted on 40  $\mu$ m steel wires in a Mulvany myograph for the measurement of isometric tension. The endothelium was removed using a human hair (verified by the lack of dilator response to bradykinin) and sections were allowed to equilibrate to 37°C in oxygenated Krebs' solution for 1 h before the optimal resting tension was determined using the active wall tension to length relationship. The myograph jaws were pulled apart incrementally and

the Laplace equation was used to calculate the internal diameter at which the intramural pressure was equal to 100 mmHg. Each segment was then set to 90% of the internal diameter as optimal force is obtained under these conditions (Mulvany & Halpern, 1977). The maximal force was measured three times with a potassium rich Krebs' solution (100 mM) at 15 min intervals.

**Conductance vessels** Right, left circumflex or left anterior descending CA were dissected free from surrounding tissue and cut into 4 mm rings. The rings were denuded of their endothelium using a blunt seeker (verified histologically), mounted in 5 ml organ baths (Linton Instrumentation, Norfolk, U.K.) for the measurement of isometric tension (F30 force transducers; Hugo Sachs, March-Hugstetten, Germany) and bathed in oxygenated Krebs' solution at 37°C. To obtain the optimal resting tension, 100 mM KCl was added at increasing levels of basal tension until no further increase in response was obtained.

### Agonist studies

Both conductance and resistance CA were allowed to equilibrate to their own resting tension for at least 1 h before the start of the experiment. Concentration-response curves to ET-1 (0.1–300 nM) were constructed and expressed as a percentage of the KCl response obtained prior to the start of the experiment. In the studies on the dilator compounds, a submaximal concentration of ET-1 (10 nM) was added and once the response had reached a plateau concentration-response curves to the natriuretic peptides (0.1–300 nM), SNAP (0.1–30  $\mu$ M) and DEA/NO (0.1–30  $\mu$ M), alone and in the presence of 100  $\mu$ M 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; preincubated with the tissue for 20 min), were constructed. Vessels were left until responses reached a plateau before further addition of agonist, or for a minimum of 5 min if no response was observed. Control rings of artery were contracted with 10 nM ET-1 and the tension measured over the time course of the experiment. The inactive nucleophile diethylamine (DEA; 10–30  $\mu$ M) was used as a negative control. Concentration-response curves to the vasodilators were expressed as percentage ET-1 constrictor response.

### Data analysis

Human CA are prone to spontaneous phasic contractions *in vitro*, but only tonic changes in the basal tension (upon which the spontaneous activity is superimposed) were measured in this study. EC<sub>50</sub> values were determined for each curve using the iterative curve-fitting software Fig. P (Biosoft, Cambridgeshire, U.K.). EC<sub>50</sub> values were expressed as geometric means with 95% confidence intervals (CI) and were compared using the Mann–Whitney *U*-test ( $P < 0.05$ ). All other data were expressed as arithmetic means  $\pm$  s.e.mean and were compared using Student's two-tailed *t*-test ( $P < 0.05$ ).

### Materials

ET-1, ANP, BNP and CNP (Peptide Institute Inc., Osaka, Japan) stock solutions (0.1 mM) were prepared in 0.1% acetic acid (ET-1) and distilled water (ANP, BNP, CNP) and stored

at  $-20^{\circ}\text{C}$ . DEA/NO (Alexis Biochemicals, Nottinghamshire, U.K.) was stored under nitrogen at  $-70^{\circ}\text{C}$  and dissolved in 0.01 M NaOH immediately prior to experiments. Stock solutions of ODQ (0.1 M; Tocris Bristol, U.K.) were prepared in dimethylsulphoxide. All other reagents were from Sigma-Aldrich Ltd. (Dorset, U.K.) or BDH Ltd. (Dorset, U.K.). Krebs' solution comprised (mM): NaCl 90,  $\text{NaHCO}_3$  45, KCl 5,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5,  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  1,  $\text{CaCl}_2$  2.25, fumaric acid 5, glutamic acid 5, glucose 10, sodium pyruvate 5 (pH 7.4).

## Results

### Vasoreactivity of conductance and resistance coronary arteries

Resistance CA had a mean internal diameter of  $467 \pm 23.5 \mu\text{m}$  and produced a maximal contractile force of  $1.99 \pm 0.19 \text{ mN mm}^{-1}$  to 100 mM KCl (62 segments from 26 hearts). Conductance CA produced a maximal contractile force to 100 mM KCl of  $14.62 \pm 0.86 \text{ mN mm}^{-1}$  (76 segments from 18 hearts).

### Constrictions to ET-1

ET-1 contracted all vessels tested and produced a sustained constriction in control segments. ET-1 contracted resistance CA significantly more potently than conductance CA ( $P < 0.05$ , Mann-Whitney *U*-test; Figure 1) with  $\text{EC}_{50}$  values of 2.98 nM (95% CI: 1.49–5.95 nM;  $n = 6$ ) and 8.58 nM (95% CI: 4.72–15.6 nM;  $n = 9$ ) respectively. The maximal response ( $E_{\text{MAX}}$ ) to ET-1 was  $140 \pm 8.22\%$  KCl response in resistance CA and  $129 \pm 25.9\%$  KCl response in conductance CA.

### Reversal of ET-1 induced constrictions by NO

In resistance CA, the NO-donor SNAP (0.1–30  $\mu\text{M}$ ) only partially reversed ( $E_{\text{MAX}}$   $60.5 \pm 11.7\%$ ;  $n = 9$ ) a constriction induced by a sub-maximal concentration of 10 nM ET-1

(Figure 2; Table 1). In contrast, constrictions induced by the same concentration of ET-1 in conductance CA were completely reversed by SNAP, and there was also an additional relaxation to below the original baseline ( $E_{\text{MAX}}$   $125 \pm 11.5\%$ ;  $P < 0.05$ , Student's *t*-test;  $n = 6$ ). The potency of SNAP was more variable in resistance CA, yielding an  $\text{EC}_{50}$  value of 1.13  $\mu\text{M}$  (95% CI: 0.25–5  $\mu\text{M}$ ) compared to 0.1  $\mu\text{M}$  (95% CI: 0.01–0.89  $\mu\text{M}$ ) in conductance CA (Table 1).

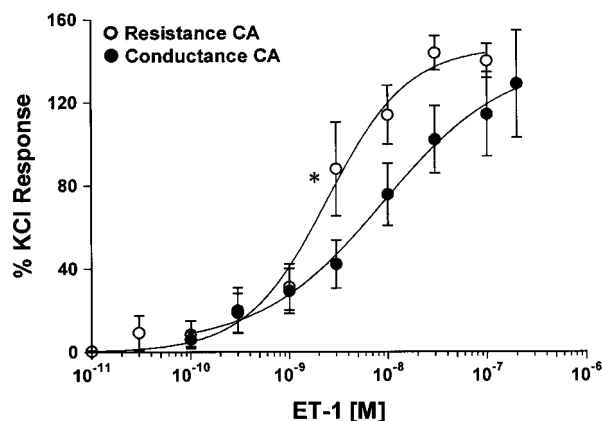
The NO-donor DEA/NO was also used to study the effects of NO in human CA as the breakdown product DEA can be used as an additional negative control. Single, submaximal doses of DEA/NO produced rapid, transient relaxations to ET-1 in conductance CA (Figure 3). Cumulative concentration-response curves to DEA/NO gave similar response to SNAP in conductance CA ( $P > 0.15$ , Mann-Whitney *U*-test; Figures 4 and 5A, Table 1;  $n = 12$ ). DEA/NO was more potent in resistance CA, although, as with SNAP the  $\text{EC}_{50}$  values were variable ( $n = 8$ ; Table 1).

### Inhibition of soluble guanylate cyclase (sGC)

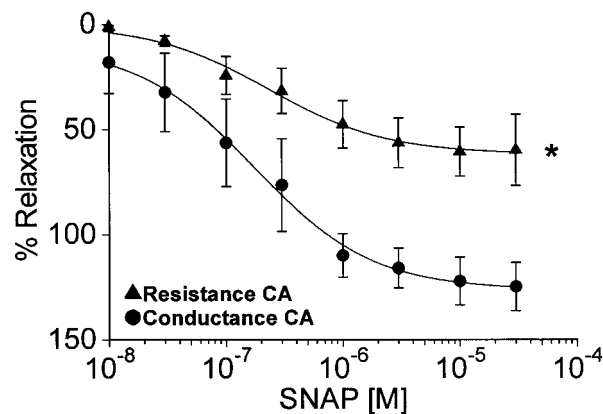
Cumulative concentration-response curves to DEA/NO were repeated in segments of CA that had been pre-incubated with the sGC inhibitor ODQ (Figure 5A). In conductance CA the maximum response to DEA/NO was significantly reduced in the presence of ODQ ( $E_{\text{MAX}}$   $76.9 \pm 14.4\%$ ;  $P < 0.05$ , Student's *t*-test;  $n = 7$ ), however ODQ had no effect on relaxations to the NO-donor in resistance CA (Figure 5B;  $E_{\text{MAX}}$   $77.2 \pm 18.4\%$ ;  $n = 3$ ).

### Reversal of ET-1 induced constrictions by natriuretic peptides

Cumulative concentration-response curves to three natriuretic peptides, ANP, BNP and CNP were constructed in conductance and resistance CA after constrictions were elicited with 10 nM ET-1 (Figure 6). Concentration-response curves to CNP did not always reach a maximum in the concentration range tested and neither did the response curves to BNP in some resistance CA. Consequently mean  $\text{EC}_{50}$  and  $E_{\text{MAX}}$  values could not be calculated in these cases. ANP dilated both conductance and resistance CA with



**Figure 1** Cumulative concentration-response curves for ET-1 in human conductance and resistance coronary arteries (0.01–100 and 0.1–200 nM;  $n = 6$  and 9 respectively).  $\text{EC}_{50}$  values for ET-1 are significantly different in resistance compared to conductance CA (\* $P < 0.05$ , Mann-Whitney *U*-test).

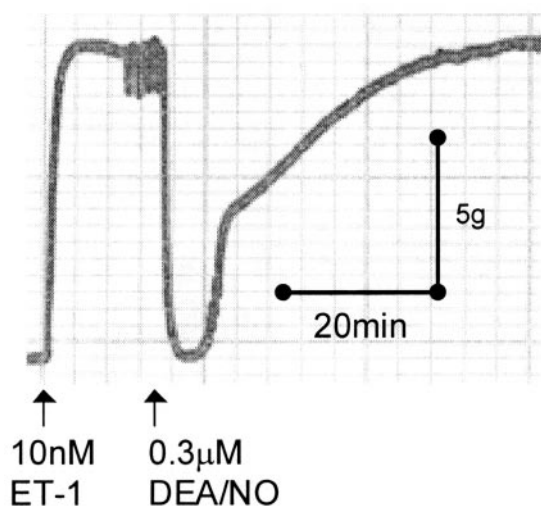


**Figure 2** Cumulative concentration-response curves to the NO-donor SNAP in human conductance and resistance coronary arteries ( $n = 6$  and 9 respectively; \* $P < 0.05$ , Student's *t*-test).

**Table 1** Effect of ANP, BNP and CNP and the NO-donors SNAP and DEA/NO on constrictions induced by ET-1 in human conductance and resistance coronary arteries

	Conductance CA			Resistance CA		
	$EC_{50}$	$E_{MAX}$ (% ET-1 response)	n	$EC_{50}$	$E_{MAX}$ (% ET-1 response)	n
SNAP	0.10 $\mu$ M (0.01–0.89 $\mu$ M)	125 $\pm$ 11.5	6	1.1 $\mu$ M (0.26–5.0 $\mu$ M)	60.5 $\pm$ 11.7*	9
DEA/NO	0.10 $\mu$ M (0.02–0.31 $\mu$ M)	127 $\pm$ 9.16	12	0.18 $\mu$ M (0.03–0.10 $\mu$ M)	78.8 $\pm$ 8.13*	8
ANP	4.25 nM (0.84–21.4 nM)	53.1 $\pm$ 14.7	7	18.4 nM (2.92–116 $\mu$ M)	48.6 $\pm$ 11.8	6
BNP	2.40 nM (0.74–7.75 nM)	54.5 $\pm$ 14.9	7	ND	ND	4
CNP	ND	ND	6	ND	ND	6

$EC_{50}$  values are expressed as geometric means with 95% confidence intervals and  $E_{MAX}$  values (the maximal response to vasodilator as a percentage of the constrictor response to 10 nM ET-1) as mean  $\pm$  s.e.mean. \* $P$  < 0.05, Student's *t*-test. ND, not determined;  $E_{MAX}$  and  $EC_{50}$  values could not be determined because concentration-response curves failed to reach a maximum at the concentration tested.

**Figure 3** An original trace recording showing a constriction to 10 nM ET-1 and subsequent transient reversal with 0.3  $\mu$ M DEA/NO in human conductance coronary artery.

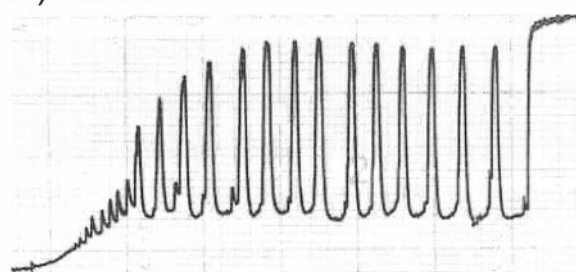
nanomolar potency (Table 1).  $E_{MAX}$  values for ANP were 53.1  $\pm$  14.7% ( $n$  = 7) and 48.6  $\pm$  11.8% ( $n$  = 6), in conductance and resistance CA respectively.

Responses to BNP only reached a plateau in conductance CA, yielding an  $EC_{50}$  value of 2.4 nM (95% CI: 0.74–7.75;  $n$  = 7) and an  $E_{MAX}$  of 54.5  $\pm$  14.9%. Concentration-response curves to CNP were not completed in either conductance or resistance CA.

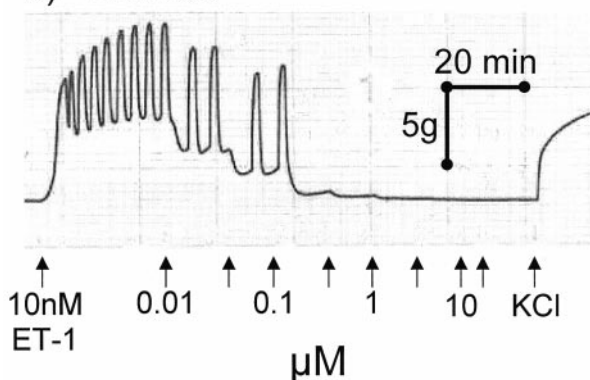
## Discussion

The spontaneous activity of human coronary arteries has been well characterized in normal, diseased and cadaveric tissue *in vitro* (Ross *et al.*, 1980; Godfraind *et al.*, 1984; Stork & Cocks, 1994). Phasic contractions can develop at any stage of the experiment and are not caused by the addition of constrictor agonists. Activation of voltage operated calcium channels has been shown to mediate the

### A) DEA



### B) DEA/NO

**Figure 4** Original trace recording showing concentration-response curves to DEA/NO and the inactive breakdown product DEA in sections of human conductance coronary artery. The phasic contractions seen are spontaneous activity of the tissue.

phasic contractions as they can be removed by the addition of calcium channel blockers such as nifedipine (Godfraind *et al.*, 1984; Stork & Cocks, 1994). However, as a component of the response to ET-1 is dependent on the influx of calcium through these channels, the addition of nifedipine in the present study would have blunted the response of the arteries to ET-1 (Pierre & Davenport, 1999). The phasic contractions are superimposed on tonic changes in tension and in the present study, only tonic changes in tension have been measured.

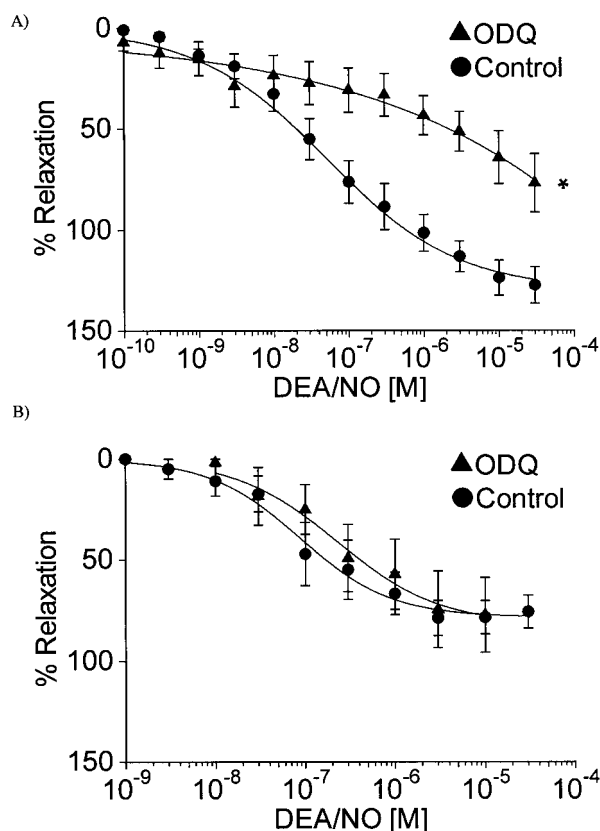
ET-1 produced a prolonged contraction in all vessels tested, with contractions in control segments typically lasting over 3 h. In contrast single, submaximal concentrations of NO caused a rapid but transient relaxation, and only

cumulative concentration curves caused a sustained dilatation. ET-1 was significantly more potent in resistance compared to conductance CA, and the  $EC_{50}$  values derived were similar to those previously published in CA of similar internal diameters (Maguire *et al.*, 1997; Pierre & Davenport, 1998).  $ET_A$  receptors predominate in the coronary vasculature and mediate constriction (Maguire & Davenport, 1995; Pierre & Davenport, 1998). *In vivo*, infusions of the  $ET_A$  antagonist BQ123 in humans caused greater vasodilatation in distal epicardial CA and resistance arterioles than proximal CA (Kyriakides *et al.*, 2000), providing further evidence to support the hypothesis that ET receptors are more sensitive in the distal coronary artery tree.

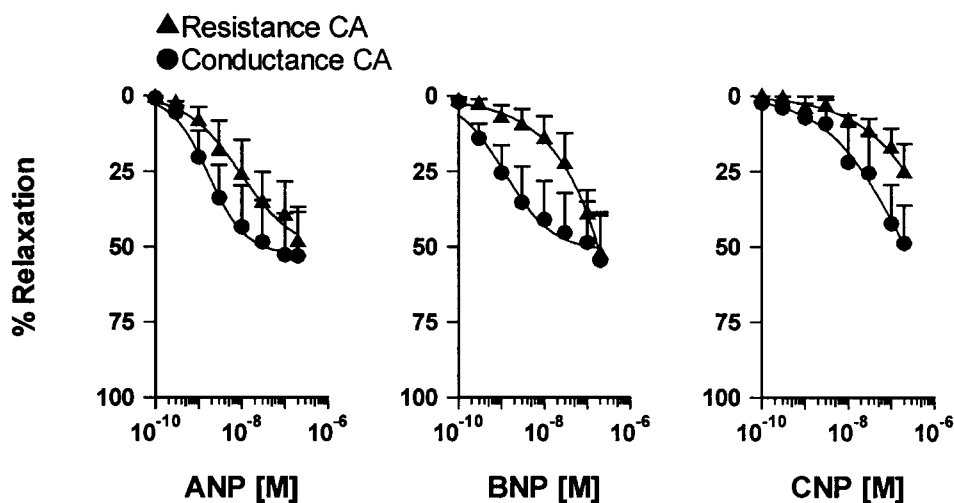
The NO donor SNAP was found to cause significantly greater vasodilatation against ET-1 constrictions in conductance compared to resistance arteries. The vasodilator action of DEA/NO is closely correlated to the spontaneous generation of NO (Morley & Keefer, 1993), thus, DEA/NO was used to more accurately mimic the continuous release of NO from the endothelium *in vivo*. Similar responses to those obtained with SNAP were seen in both vessel types; there was complete reversal of ET-1-mediated constrictions in conductance CA, with an additional relaxation of the basal tone, but only partial reversal in resistance CA.

*In vivo* infusions of NOS inhibitors in healthy human volunteers show that NO contributes to resting tone in both conductance and resistance CA, but that inhibition of NOS has a greater effect in the larger CA (Quyyumi *et al.*, 1995).

In order to assess the extent to which NO-mediated vasodilatation was dependent on cyclic GMP in conductance CA, concentration-response curves to DEA/NO were repeated in the presence of the sGC inhibitor ODQ. Surprisingly, the maximum response to DEA/NO was only reduced by approximately half, even in the presence of 100  $\mu$ M ODQ, a concentration which we have previously shown to completely block cyclic GMP production in human tissue (Wiley & Davenport, 2001). Intriguingly, sGC inhibition had no effect on relaxations to DEA/NO in resistance CA. The lack of effect of ODQ on resistance CA, coupled with the fact that in conductance CA, ODQ reduced



**Figure 5** Cumulative concentration-response curves to DEA/NO alone and in the presence of 100  $\mu$ M ODQ (A) Human conductance coronary artery ( $n=12$  and  $7$ ;  $*P<0.05$ , Student's *t*-test, maximum control response compared to maximum response in the presence of ODQ) (B) Human resistance coronary artery ( $n=8$  and  $3$ ).



**Figure 6** Cumulative concentration-response curves to natriuretic peptides in human conductance and resistance coronary arteries. ANP ( $n=7$  and  $6$ ) BNP ( $n=7$  and  $4$ ) CNP ( $n=4/6$  and  $6$ ).

the maximum response to NO to a similar level to that seen in resistance vessels, suggests that the difference between the two vessel types could be that small vessels do not express sGC. An alternative explanation is that resistance CA are much less sensitive to cyclic GMP. Further studies would be required to test these hypotheses, however the measurement of cyclic GMP in resistance CA is not possible at present as the levels generated would be below the level of detection.

The large non-cyclic GMP-dependent component of the NO induced vasodilatation could be mediated *via* several pathways. For example NO could be directly activating potassium channels such as calcium-dependent potassium channels (Bychkov *et al.*, 1998) or ATP-sensitive potassium channels (Miyoshi *et al.*, 1994). Alternatively, NO could be directly modifying the ET receptor to reduce the level of ET-1 binding (Wiley & Davenport, 2001).

The present study also compared the ability of the natriuretic peptides to reverse ET-1 mediated constrictions by directly acting on the vascular smooth muscle layer. ANP partially reversed constrictions to ET-1, with similar potency and maximal response in both vessel groups. Previous studies have presented mixed findings regarding the relative potency of ANP in the coronary vasculature. Single concentrations of ANP partially reversed potassium-induced constrictions in human conductance CA *in vitro* (Rapoport *et al.*, 1986). *In vivo* infusions in normotensive individuals and patients coronary artery disease have found ANP to cause vasodilatation in conductance but not resistance CA (Herrmann *et al.*, 1990; Valsson *et al.*, 1996). In contrast Egashira *et al.* (1991) reported that in addition to dilating epicardial CA, there was an increase in coronary blood flow in healthy volunteers, indicating dilatation of the resistance vasculature. Another study in healthy humans measured sustained dilations in proximal CA and transient increases in coronary flow which lasted less than 5 min after a bolus dose of ANP (Chu *et al.*, 1989). Taken together these results suggest that ANP causes prolonged dilatation in conductance CA, and a transient dilatation in resistance CA where the effects are rapidly reversed *in vivo*, perhaps by the counter-regulatory action of baroreceptors.

BNP reversed ET-1-induced constrictions in conductance vessels with similar magnitude and potency to ANP. BNP may have a greater effect on resistance arteries *in vivo* because part of the vasodilatation seen is endothelium-dependent activation of NOS and cyclo-oxygenase (Zellner *et al.*, 1999). Indeed, the response to BNP in resistance CA was nearly abolished in the presence of L-NAME, an effect not observed

in the epicardial CA, and as the present study was conducted on endothelium denuded arteries, this may account for the 6 fold greater potency in conductance CA compared to resistance CA.

CNP caused very little vasodilatation in either vessel type at the concentration tested. This is surprising as CNP, unlike ANP and BNP, is only synthesized in the endothelium and is not thought to have any natriuretic properties. Studies have shown that CNP stimulates cyclic GMP production in cultured human vascular smooth muscle cells from internal mammary arteries (Ikeda *et al.*, 1996; Zhang *et al.*, 1996) and 1  $\mu$ M CNP caused greater than 60% reversal of human internal mammary artery pre-constricted with ET-1 (Protter *et al.*, 1996). However systemic infusion of CNP in humans causes a reduction in diastolic and systolic blood pressure only at a dose approximately 50 times greater than the physiological plasma level (Igaki *et al.*, 1996). Previously, the lack of an up-regulation in plasma CNP in patients with chronic heart failure has been attributed to the paracrine, rather than endocrine nature of CNP (Wei *et al.*, 1993), but the lack of vasodilator potency seen in this study indicates that the main role of CNP in the coronary vasculature is yet to be determined.

In conclusion, the greater potency of ET-1 in resistance vessels may exacerbate the effects of increased circulating levels of the peptide in disease. Whilst NO will reverse ET-1 mediated constrictions in conductance CA, neither of the NO-donors tested could completely reverse constrictions in resistance CA. None of the natriuretic peptides could fully reverse ET-1 in the coronary vasculature and BNP was found to have heterogeneous effects on the vascular smooth muscle of conductance and resistance CA. Other mediators such as endothelium-derived hyperpolarizing factors or prostanoids may also contribute to the maintenance of dilator tone, however these results would suggest that *in vivo* the constrictor tone generated by ET-1 is opposed by a combination of dilator molecules. An effective therapy to counteract ET-1 in resistance CA in pathophysiological conditions may be an ET<sub>A</sub> receptor antagonist, particularly as the endothelium is dysfunctional in cardiovascular disease.

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