British Journal of Pharmacology (1998) 124, 499–506
http://www.stockton-press.co.uk/bjp

Endothelin receptor subtypes and their functional relevance in human small coronary arteries

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1 The potent constrictor peptide endothelin (ET) has been implicated in various cardiovascular disorders including myocardial infarction and atherosclerosis. We have investigated the nature of ET receptor subtypes present on human small coronary arteries.

2 Small coronary arteries were mounted in a wire-myograph for *in vitro* pharmacology. To investigate the ET receptor subtypes present in different segments of the coronary vascular tree, arteries were grouped according to internal diameter. Responses in arteries with small internal diameters (mean 316.7 ± 7.9 μm; Group B) were compared to those in larger arteries (mean 586.2 ± 23.1 μm; Group A).

3 ET-1 consistently and potently contracted arteries from Group A and B, with EC_{50} values of 1.7 (0.9–3.2) nM ($n=15$) and 2.3 (1.4–4.2) nM ($n=14$), respectively. No correlation was observed between ET-1 potency and internal diameter. The response to ET-1 was potently antagonized by the selective ET_A receptor antagonist PD156707 in both Group A and Group B, yielding pA_2 values of 8.60 ± 0.12 ($n=4-6$) and 8.38 ± 0.17 ($n=4-6$), respectively. Slopes from Schild regression were not significantly different from unity.

4 In contrast to ET-1, individual responses to ET-3 were variable. While all arteries from Group A responded to ET-3 ($EC_{50} \sim 69$ (23–210) nM) ($n=12$), no response was obtained in 5 of the 14 tested in Group B. Of those responding, many failed to reach a maximum at concentrations up to 1 μM. ET-1 was more potent than ET-3 in all arteries tested. A biphasic ET-3 response was observed in 8 arteries suggesting that a small ET_B population was also present in some patients. The selective ET_B receptor agonist sarafotoxin S6c had little or no effect up to 10 nM ($n=4-6$).

5 Responses to ET-1 and ET-3 were unaffected by removal of the endothelium in arteries from both groups suggesting a lack of functional, relaxant ET_B receptors on endothelial cells ($n=5$).

6 Using autoradiography, specific high density binding of the non-selective, ET_A/ET_B ligand [^{125}I]-ET-1 and selective ET_A ligand [^{125}I]-PD151242 was detected on the vascular smooth muscle layer of small intramyocardial coronary arteries ($n=5$). In contrast, little or no binding of the selective ET_B receptor ligand [^{125}I]-BQ3020 was observed ($n=5$). Similarly, [^{125}I]-ET-1 binding to vascular smooth muscle was absent in the presence of the selective ET_A receptor antagonist PD156707.

7 We conclude that human small epi- and intramyocardial coronary arteries express predominantly ET_A receptors and it is these receptors which mediate ET-induced contractions. A constrictor ET_B receptor population may exist in some patients. However, these receptors may have a limited role as contractions to ET-1 can be blocked fully by the selective ET_A receptor antagonist PD156707.

Keywords: Endothelin; ET_A and ET_B receptor; human small coronary artery; *in vitro* pharmacology; autoradiography; PD156707; [^{125}I]-PD151242; [^{125}I]-BQ3020

Introduction

The potent constrictor action of endothelin-1 (ET-1) in both human and animal coronary vasculature is now well documented (Yanagisawa *et al*., 1988; Franco-Cereceda, 1989; Chester *et al*., 1992; Godfraind, 1993; Opgaard *et al*., 1994; Maguire & Davenport, 1995). This potency, taken together with the unusually sustained action of ET, suggests a role for the peptide in the pathogenesis of various vascular disorders including coronary vasospasm and myocardial ischaemia.

Atheromatous plaques within the coronary arteries (coronary artery disease) may reduce perfusion of the myocardium leading to ischaemic damage or in cases of complete occlusion myocardial infarction. Increased ET levels have been described, not only in the plasma of patients with advanced atherosclerosis (Lerman *et al*., 1991; Cassone *et al*., 1996), but also within the atherosclerotic tissue itself (Zeiher *et al*., 1995; Bacon *et al*., 1996). Similarly, cultured smooth muscle cells from atherosclerotic coronary arteries secreted significantly more ET compared to controls (Haug *et al*., 1996). Atherosclerotic lesions appear to be present mainly in large epicardial coronary arteries with limited, if any, disease being observed in small or intramyocardial arteries. Although the small vessels may be spared of disease, they are downstream of the larger atherosclerotic vessels and thus could be exposed to increased levels of ET, which may lead to functional changes.

Raised ET levels have also been associated with ischaemic conditions. In a pig model of myocardial ischaemia, increased levels of ET have been described in ischaemic myocardial tissue compared to non-ischaemic (Wang *et al*., 1995), while plasma ET levels are raised in patients following myocardial infarction (Miyauchi *et al*., 1989; Yasuda *et al*., 1990). Thus, small intramyocardial vessels may be exposed to increased local production of ET by the ischaemic tissue itself. Chronic exposure to elevated ET levels may then cause blunting of the response to ET due to downregulation of receptors (Schiffrin *et al*., 1992). Alternatively, there may be a general increase in coronary vascular tone, further reducing blood flow to the

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myocardium. Under such conditions dilatation of small coronary arteries may be of particular importance.

In larger, muscular coronary arteries (internal diameter > 1mm) ET-induced constriction is mediated predominantly via the ET_A receptor subtype with a small and variable contribution from ET_B receptors (Maguire & Davenport, 1995). However, there has been some suggestion of changes in ET receptor expression along the coronary vascular tree. A study by Godfraind (1993) suggested that ET_A receptors mediate constriction in smaller, pre-resistant coronary arteries, while more than one ET receptor is involved in the larger segments. In contrast, a study by Dashwood and colleagues (1995), indicated an increase in ET_B binding sites in smaller coronary arteries compared to larger vessels. In addition, in man, vasoconstrictor ET_B receptors have been described particularly in small resistance arteries. While small arteries from human peripheral and pulmonary vascular beds have been studied (Tshcudi & Luscher, 1994; Haynes *et al*., 1995; Deng *et al*., 1995; McCulloch *et al*., 1996), little is known about the ET receptor subtypes mediating constriction in small coronary arteries.

It was therefore, of interest to determine which ET receptor subtypes are expressed and importantly, which are of functional relevance in human small coronary arteries, particularly if ET receptor antagonists are to be of therapeutic use in coronary vascular disorders.

As an initial step, we have investigated whether the potency of ET-1 varied according to artery diameter. Given the possibility of changes in ET receptor expression along the coronary vascular tree, we then grouped arteries according to internal diameter (i.d.). In doing this, we aimed to identify a group of small arteries which were more likely to contribute to coronary resistance. It is these arteries which are directly involved in the control of blood flow to the myocardium and are therefore of particular importance. A preliminary account of this work has been presented to the British Pharmacological Society (Pierre & Davenport, 1996; 1997).

Methods

Heart apices were obtained from 35 patients (27 male, 8 female; mean age 46.0 ± 2.1 years) undergoing cardiac transplantation for ischaemic heart disease, cardiomyopathy or valve disease. One patient was undergoing a heart-lung transplant. Patient drug therapy included diuretics, anti-coagulants, anti-hypertensives, vasodilators and anti-arrhythmics. Tissue collection was carried out from Papworth Hospital, U.K., with local ethical committee approval. Heart apices were obtained at the time of surgery and placed in cold Krebs solution. A portion of the each apex was frozen and stored at -70°C until used for sections.

Autoradiography

Slide mounted cryostat sections (10 μm) of human heart apex were pre-incubated with buffer (50 mM HEPES, 5 mM $MgCl_2$, 0.3% bovine serum albumin, pH 7.4) for 15 min. Adjacent sections were then incubated, for 2 h, in buffer containing either [^{125}I]-PD151242 (0.1 nM), to label ET_A receptors or [^{125}I]-BQ3020 (0.3 nM), to label ET_B receptors (Davenport *et al*., 1994). By use of the law of mass action, the concentrations of ligands used have been calculated to label $\sim$30% of the respective receptor populations in human coronary artery (Bacon & Davenport, 1996). In addition, [^{125}I]-ET-1 (0.1 nM) binding was visualized in the presence of PD156707 (50 nM). At this concentration, it was determined that PD156707 would block $\sim$98% of ET_A receptors and $\sim$3% of ET_B receptors (Maguire *et al*., 1997). Non-specific binding was determined by inclusion of the corresponding unlabelled ligand (1 μM). Sections were washed in ice-cold Tris-HCl buffer, dried and apposed to radiation sensitive-film (Hyperfilm β-max) for 5 days at 22°C.

In vitro *pharmacology*

By use of a dissecting microscope, small coronary arteries were dissected from the surface of the apex, often just before they enter the myocardium, and cleaned of any adhering fat. Rings of artery (1–2 mm in length) were threaded onto 40 μm diameter stainless steel wires and mounted onto jaws within a wire-myograph, (Model 500A; J.P.Trading, Aarhus, Denmark), containing oxygenated (95% O_2: 5% CO_2) modified Krebs solution, (composition (mM): NaCl 90, KCl 5, $MgSO_4$. $7H_2O$ 0.5, Na_2HPO_4 1, $NaCO_3$ 45, $CaCl_2$ 2.25, glucose 10, glutamate 5, Na pyruvate 5, fumarate 5 and EDTA 0.04, pH 7.4) maintained at 37°C. Isometric tension measurements were made via force transducers mounted on the myograph jaws. Output was displayed digitally on the myograph and on a Graphtec chart recorder (Linton Instrumentation, Diss, U.K.). Following a 1 h equilibrium period, the vessels were stretched radially and the wall tension to internal circumference relation determined. By use of the Laplace relationship, the internal diameter (i.d.) at which the transmural pressure was 100 mmHg (i.e. as it would be, when relaxed, *in vivo*) could be estimated (Mulvany and Halpern, 1977). The vessels were then set to 90% of this i.d. as under these conditions maximal contractile force is obtained (Mulvany and Halpern, 1977).

Vessels were stimulated twice with a potassium rich solution (95 mM) to assess contractile function. Subsequently, a contraction to the thromboxane A_2 mimetic U46619 (300 nM) was obtained. On plateau of the contraction, bradykinin (100–300 nM) was administered to test for a functional endothelium. For some experiments the endothelium was removed by gently rubbing the luminal surface of the arterial ring with a human hair. Following a 45 min recovery period the vessels were re-challenged with U46619 and the test for functional endothelium was repeated. Successful removal of the endothelium was confirmed by the loss of bradykinin-induced relaxation.

Agonist studies

Cumulative concentration-response curves were constructed to either ET-1 (1 pM–300 nM), ET-3 (1 pM–700 nM) or the selective ET_B receptor agonist, sarafotoxin S6c (1 pM–700 nM). Responses were expressed as a % of the potassium-induced contraction. One curve was constructed per preparation. Concentration-response curves were analysed by use of the curve fitting package Fig. P. (Biosoft, Cambridge, U.K.), to determine the EC_{50} (the concentration required to produce 50% of the maximal response) for agonists in each preparation.

Antagonist studies

Cumulative concentration-response curves (CRC) to ET-1 (1 pM–300 nM) were constructed in the absence or presence of increasing concentrations of the selective, non-peptide, ET_A receptor antagonist, PD156707 (10, 30, 100 nM) (Reynolds *et al*., 1995). PD156707 was added 30 min before the construction of the ET-1 CRC. Antagonist potency, expressed as a pA_2, was determined by Schild regression (Arunlakshana & Schild, 1959).

Materials

[^{125}I]-ET-1, [^{125}I]-PD151242, [^{125}I]-BQ3020 (all $\sim$2000 Ci mmol^{-1}) and Hyperfilm β-max was purchased from Amersham Pharmacia Biotech (Amersham, U.K.). ET-1, ET-3 and S6c were purchased from the Peptide Institute (Osaka, Japan). Stock solutions (100 μM) were made by dissolving the peptides in 0.1% acetic acid and kept frozen at -20°C until needed Unlabelled BQ3020 ([Ala^{11,15}]Ac-ET-1$_{(6\text{-}21)}$) was synthesized by solid-phase t-boc chemistry. PD156707 (sodium 2-benzo(1,3)dioxol-5-yl-4-(4-methoxy-phenyl) - 4 -oxo -3 - (3,45 -trimethoxybenzyl)-but-2-enoate), PD151242 (N-[(hexahydro-1-azepinyl) carbonyl]L-Leu(1-Me)D-Trp-D-Tyr) and FR139317 (N-[hexahydro-1-azepinyl) carbonyl]L-Leu(1-Me)D-Trp-3-(2-pyridyl)D-Ala) were synthesized by Parke-Davis Pharmaceuticals Research (Ann Arbour, MI, U.S.A.). PD156707 was dissolved in dimethylsulphoxide (DMSO) and FR139317 in distilled water to give a 10 mM stock solutions. Stock solution aliquots were frozen at -20°C and diluted, in either DMSO or water, as needed. All other peptides and reagents were from Sigma Chemical Co. (Poole, Dorset, U.K.) or BDH (Lutterworth, Leics., U.K.).

Statistical analysis

All *n* values refer to number of individual patients from which arteries were obtained. EC_{50} values are given as geometric means with 95% confidence intervals. EC_{50} values were compared for significance difference by the Mann-Whitney U test ($P<0.05$). Internal diameter and E_{max} values are arithmetic means with s.e.mean. Significant difference between Schild regression slopes and unity was tested by use of the one sample Student's *t* test ($P<0.05$).

Results

Autoradiography

Specific high density binding of [^{125}I]-ET-1 and [^{125}I]-PD151242 was detected on the vascular smooth muscle layer of intramyocardial coronary arteries. In contrast, little or no [^{125}I]-BQ3020 binding was observed (Figure 1). In addition, little specific [^{125}I]-ET-1 binding remained in the presence of the selective ET_A receptor antagonist PD156707 (50 mM (Figure 1).

In vitro *pharmacology*

Agonist studies: all arteries Human small coronary arteries contracted in response to KCl and U46619 (300 nM) with a mean absolute tensions of 3.2 ± 0.2 and 3.6 ± 0.2 mN mm^{-1}, respectively. Vasodilator responses to bradykinin (BK;

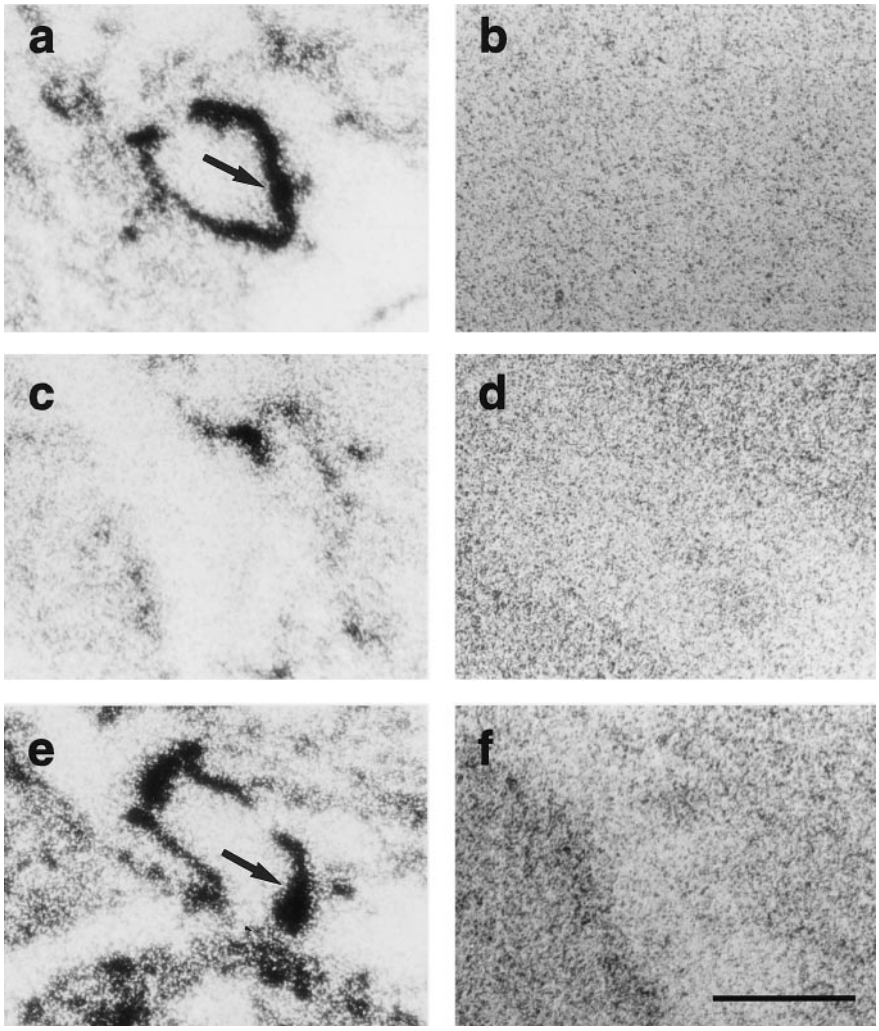

Figure 1 Representative autoradiogram showing binding of [^{125}I]-PD151242 (a), [^{125}I]-BQ3020 (c) and [^{125}I]-ET-1 in the absence (e) and presence of 50 nM PD156707 (f). Non-specific binding is shown for [^{125}I]-PD151242 and [^{125}I]-BQ3020 in (b) and (d), respectively. Scale bar represents 350 μm, arrow head = intramyocardial blood vessel.

76.4±2.6% relaxation of the U46619-induced contraction) were obtained in 141 of the 151 unrubbed segments tested and were similar in arteries from Group A and B.

ET-1 potently constricted all coronary arteries tested. The response was monophasic, yielding an EC_{50} of 2.0 nM (1.3–2.9 nM) and E_{max} of 127.5±7.5%. In contrast, responses to ET-3 were more variable. Of the 26 arteries tested, 5 failed to respond to ET-3. In vessels which did respond to ET-3, E_{max} values ranged from 13.1% to 163.8 %. A mean EC_{50} for ET-3 could only be estimated from the fitted curves by extrapolation (~56 nM (2.3–140 nM)) as some responses failed to reach a maximum within the concentration range tested. Some responses to ET-3 appeared to be biphasic in nature; this was observed in 4 arteries from each group. S6c failed to elicit a response in 9 of 10 arteries tested. A small response was observed in one artery (EC_{50} 0.3 nM; E_{max} 26.3%).

The possibility of changes in ET-1 potency with artery diameter was investigated by regression. No correlation between artery i.d. and ET-1 potency (EC_{50}) was observed ($r=0.17$). The lack of effect or failure to reach a maximum response to ET-3 in some arteries made any correlation of the potency of this peptide and internal diameter difficult.

In order to investigate the possibility of changes in ET receptor expression along the coronary vascular tree, the arteries were split into two groups according to internal diameter (i.d.). Group A had a mean i.d of 586.2±23.1 μm and Group B 316.7±7.9 μm.

Group A ET-1 potently contracted arteries from Group A with an EC_{50} of 1.7 (0.9–3.2) nM. ET-3 was less potent than ET-1 yielding an estimated EC_{50} of ~69 (23–120) nM (Table 1; Figure 2). S6c failed to elicit a response in 4 arteries tested.

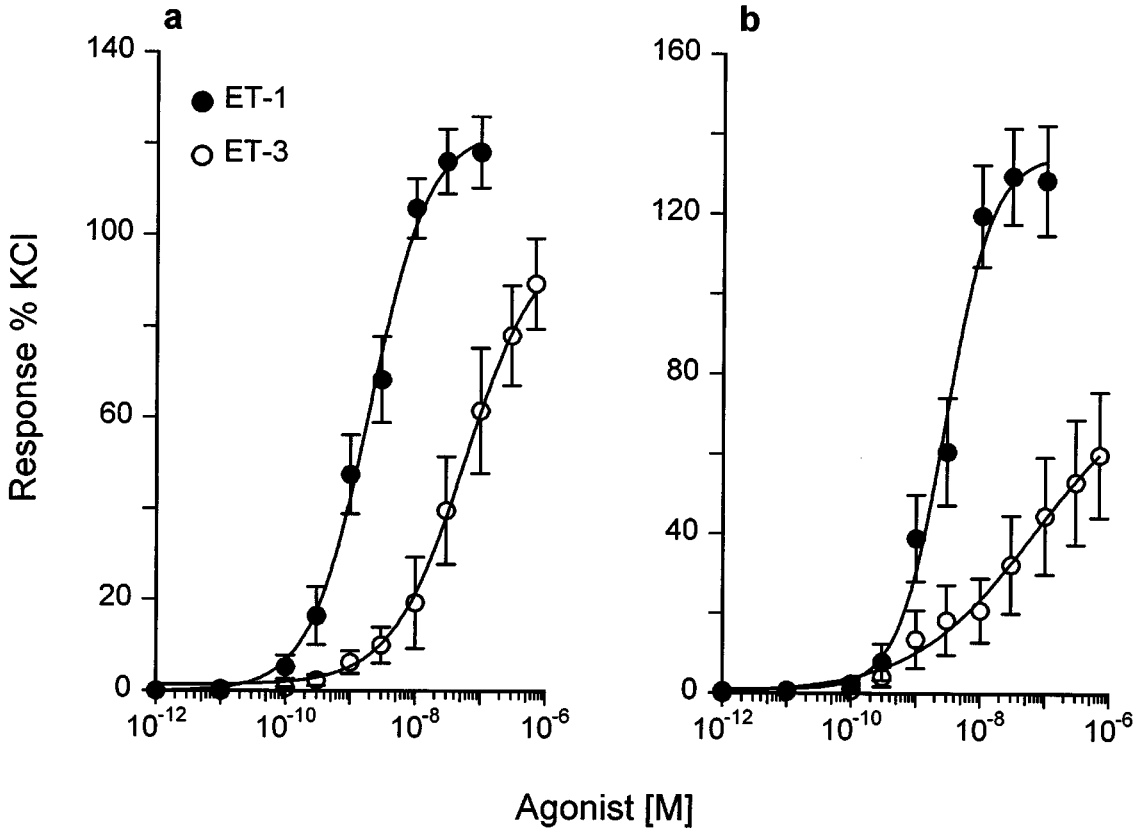

Figure 2 Concentration-response curves for ET-1 and ET-3 in human coronary artery. Vessels are grouped according to internal diameter (i.d.): (a) Group A (i.d. >400 μm; $n=15$ and 12 for ET-1 and ET-3 respectively) and (b) Group B (i.d. <400 μm; $n=14$ and 9 for ET-1 and ET-3, respectively). Values are mean and are expressed as a % of the response to KCl; vertical lines show s.e.mean. No response to ET-3 was observed in 5 of the 14 arteries tested in Group B and values are mean of responders only.

Table 1 Effect of ET-1 and ET-3 on human small coronary artery (Group A: internal diameter greater than 400 μm and group B: internal diameter less than 400 μm)

	ET-1			*ET-3*		
	EC_{50} (nM)	E_{max} (% KCl)	n	EC_{50} (nM)	E_{max} (%KCl)	n
Group A	1.7 (0.9–3.2)	118.0±7.1	15	69 (23–210)*	90.9±9.6*	12
Group B	2.3 (1.4–4.2)	130.1±11.9	14	ND	ND	9

EC_{50} values are expressed as geometric mean with 95% confidence interval and the E_{max} (maximal contraction as a % of the response to KCl) as mean±s.e.mean. ND=not determined due to lack of response or failure to reach maximum. *As all responses to ET-3 did not reach plateau, values could only be estimated by extrapolation.

Group B ET-1 was also a potent constrictor of vessels from this group with an EC_{50} of 2.3 (1.4–4.2) nM. In contrast to Group A, responses to ET-3 in Group B were more variable with little or no response being observed in 5 of the 14 arteries tested (Figure 2). Of the remaining 9, failure to reach a maximum response to ET-3 within the concentration range tested meant that an EC_{50} could not be determined. The small response to S6c (E_{max} 26.3%) was seen in 1 of the 6 segments tested in this Group.

Relaxations to bradykinin administration (100–300 nM) were markedly reduced or abolished following rubbing of the artery intimal surface. Responses to ET-1 and ET-3 were unaffected by removal of the endothelium in arteries from both groups with similar EC_{50} values being obtained (Figure 3).

Antagonist studies

The selective ET_A receptor antagonist PD156707, caused a parallel rightward shift of the concentration-response curve to ET-1 in arteries from both Group A and B with pA_2 values of 8.60 ± 0.12 and 8.38 ± 0.17, respectively (Figure 4; Table 2). No portion of the curve was resistant to the ET_A antagonist. In some experiments the lower concentrations of PD156707 were more effective than expected, a finding which has been described previously for PD156707 in human conduit coronary artery (Maguire *et al*., 1997). The slopes obtained from Schild analysis were not significantly different from one (Figure 4; Table 2).

Discussion

In the present study we have demonstrated a predominance of ET_A receptors in human small, coronary arteries including resistance and intramyocardial vessels. The term resistance artery has been applied to vessels with varying i.d ranging from <2mm (Struyker Boudier *et al*., 1990), <800 μm (Godfraind, 1993) and <500 μm (Nyborg & Mikkelson, 1988; Mulvany & Aalkjaer, 1990). Limited data on the pressure profile of small arteries *in vivo* makes the definition of resistance arteries, in terms of internal diameter, difficult. It is likely that arteries of various diameters along the vascular tree contribute in some degree to vascular resistance. In the present study, we have made a conservative estimate grouping arteries into two categories; Group A with an i.d. of >400 μm (mean i.d. 586.2 ± 23.1 μm), and Group B, i.d. <400 μm (mean i.d. 316.7 ± 7.9 μm). From the data available, it is likely that arteries from Group B contribute to coronary resistance.

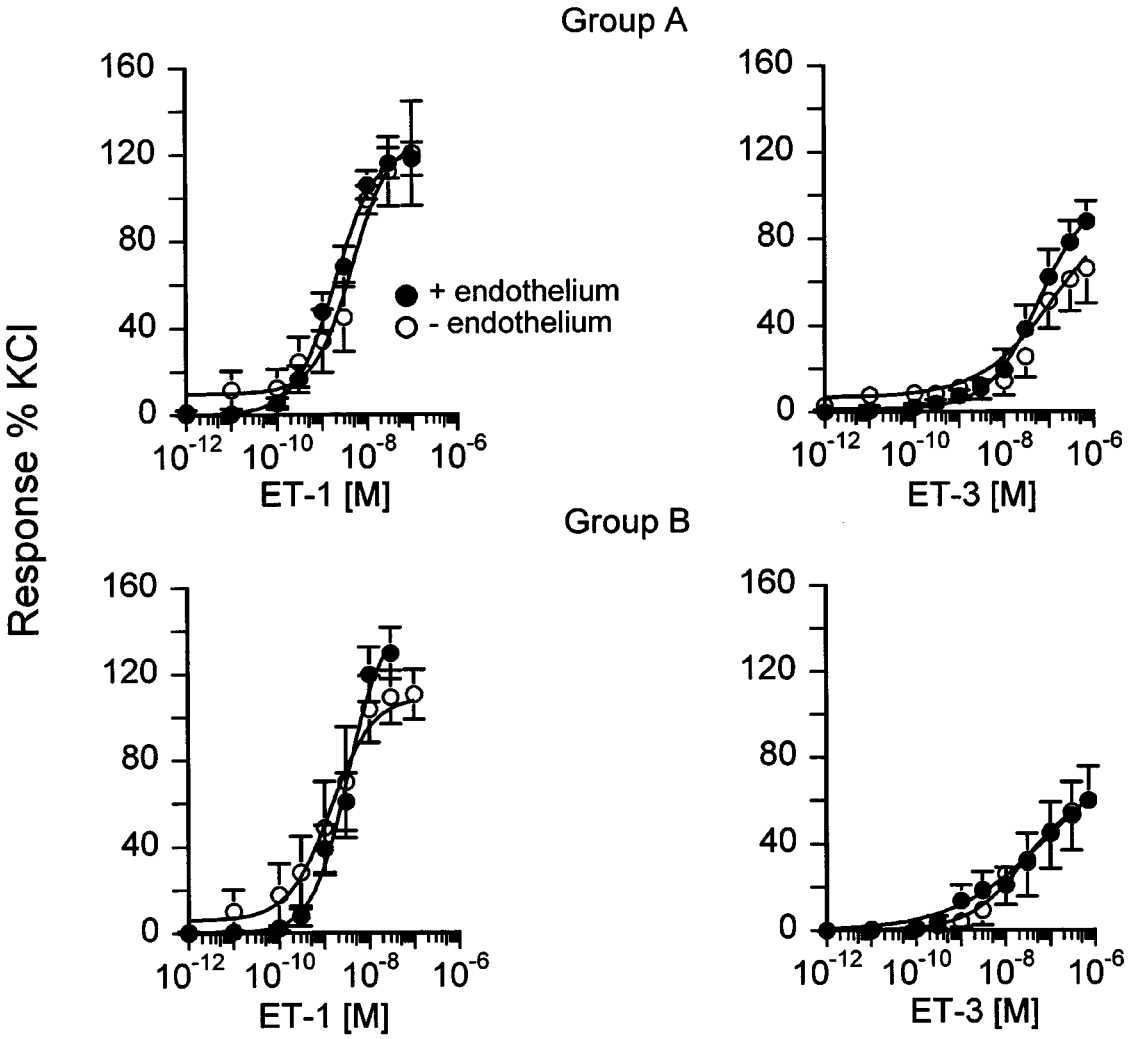

Figure 3 Concentration-response curves for ET-1 and ET-3 in arteries with and without endothelium from Group A (i.d. >400 μm; $n = 15$ and 12 for ET-1 and ET-3, respectively with endothelium and $n = 5$ for both ET-1 and ET-3 without endothelium) and Group B (i.d. <400 μm; $n = 14$ and 9 for ET-1 and ET-3 with endothelium and $n = 5$ for both without endothelium). Values are mean and are expressed as a % of the response to KCl; vertical lines show s.e.mean.

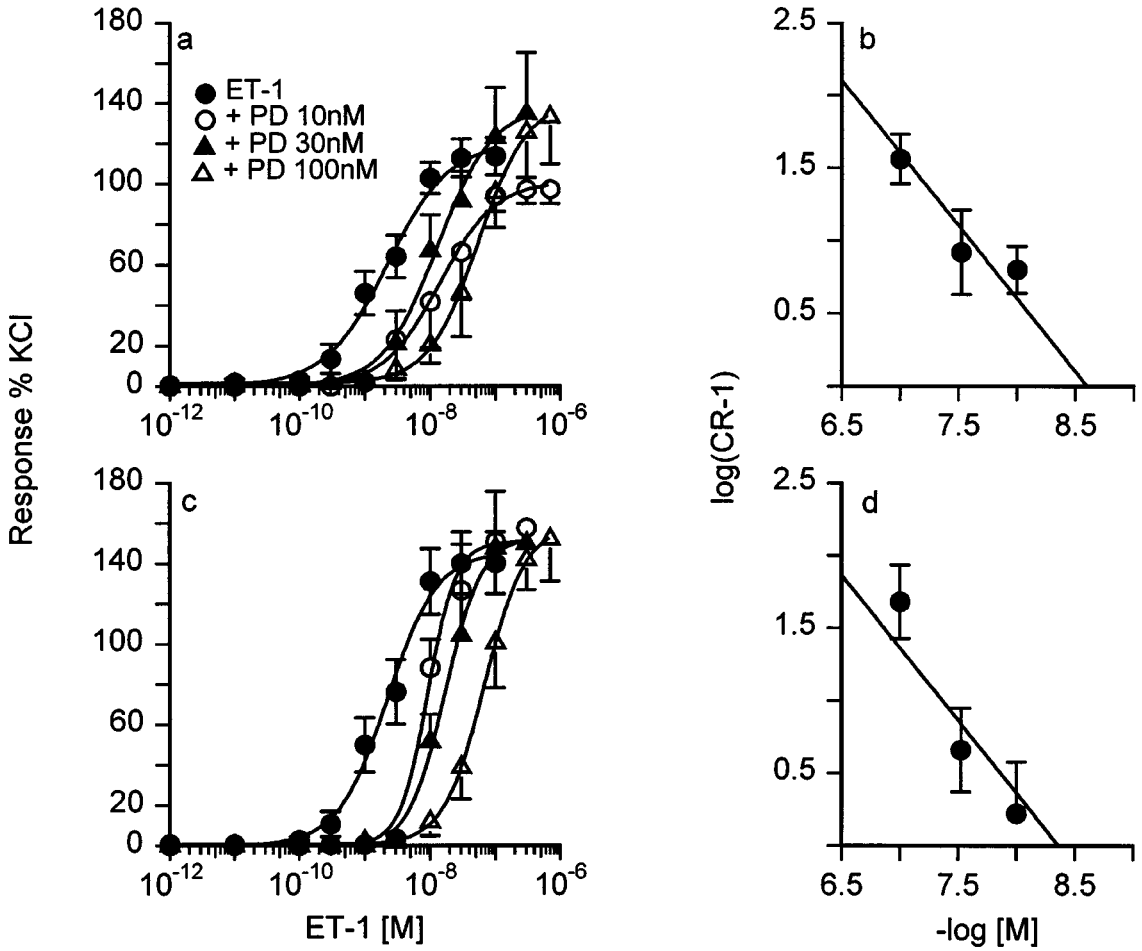

Figure 4 (a and c) Concentration-response curves for ET-1 in the absence and presence of the selective ET_A receptor antagonist PD156707, in human small coronary artery from Group A (i.d. >400 μm; $n=4$, 4, and 5 for 10, 30 and 100 nM, respectively) and Group B (i.d. <400 μm; $n=5$, 5 and 6 for 10, 30 and 100 nM, respectively). (b and d) Schild regression for antagonism of ET-1 by PD156707 in arteries from Group A and B, respectively. Values are mean and are expressed as a % of the response to KCl; vertical lines show s.e.mean.

Table 2 Effect of the nonpeptide selective ET_A receptor antagonist PD156707 (10, 30, 100 nM) on responses to ET-1 in human small coronary artery (Group A: internal diameter greater than 400 μm and Group B: internal diameter less than 400 μm)

	ET-1 EC_{50} (nM)	*pA_2*	*Negative slope*
Group A	1.4 (0.6–3.2)	8.60±0.12	0.78±0.29
Group B	2.0 (0.9–4.1)	8.38±0.17	1.48±0.41

EC_{50} values are expressed a geometric mean with 95% confidence interval. pA_2 values were determined by Schild regression. Slopes were not significantly different from one (Student's t test $P<0.05$). Values are mean data from 4–6 patients per concentration tested in both groups.

Several studies have demonstrated the potent coronary vasoconstrictor actions of ET-1 both *in vitro* and *in vivo* (Davenport *et al.*, 1989; Chester *et al.*, 1992; Pernow *et al.*, 1996). Although mRNA for both ET_A and ET_B receptors has been detected in the media of human conduit coronary artery (internal diameter >1 mm) (Davenport *et al.*, 1995), binding and functional data indicate that ET-1-induced constriction is mediated predominantly via the ET_A receptor, with a small and variable ET_B component (Opgaard *et al.*, 1994; Maguire & Davenport, 1995; Bacon & Davenport, 1996). Consistent with the findings of Godfraind (1993), we have shown a predominantly ET_A receptor population in human small distal coronary arteries. However, it should be noted that the arteries investigated in the present study have a smaller i.d. compared to those studied by Godfraind (1993) (mean 316.7 μm compared to 1.6 mm), and therefore represent arteries further along the coronary tree. Taken together, these data suggest the consistent expression of ET_A receptors in the distal portion of the coronary vasculature.

Do responses to ET-1 change according to coronary artery diameter?

ET-1 contracted arteries from both Group A (i.d. >400 μm) and B (i.d. <400 μm) with similar EC_{50} values of 1.7 nM and 2.3 nM, respectively. These values are comparable with those obtained in other human small arteries such as omental and internal mammary artery (Riezebos *et al.*, 1994; Tschudi & Luscher, 1994). Thus, the potency of ET-1 was independent of the vessel size within the range tested here. Although an increase in potency of ET-1 along the coronary vasculature has been reported (Chester *et al.*, 1992; Godfraind, 1993; Balligand & Godfraind, 1994), the vessels used in these studies had i.d.

ranging from 0.8–4 mm and were therefore, much larger than those tested in the present study. Any changes in sensitivity according to diameter may only be present in these larger arteries. Indeed, although no difference in potency was observed in the arteries tested here, the mean EC_{50} for ET-1 obtained in these small arteries is around 6 fold lower than that found for larger human coronary artery (Maguire & Davenport, 1995).

Agonist studies

Whilst all vessels in Group A responded to ET-3, responses in Group B were more variable with 5 arteries failing to respond. Evidence for variability in responses to ET-3 in human coronary artery has been described previously (Opgaard *et al*., 1994; Maguire & Davenport, 1995), although the reason for this inconsistency is unknown. Similar interpatient variability in response to vasoconstrictors such as 5-hydroxytryptamine have also been described in human small coronary artery (Angus *et al*., 1993).

The biphasic effect of ET-3 observed in some arteries suggests the involvement of more than one ET receptor subtype in these patients (i.e. both ET_A and ET_B receptors) . No correlation between this response and underlying disease (e.g. ischaemic heart disease versus cardiomyopathy) or vessel diameter was apparent.

Are functional endothelial ET_B receptors present?

The lack of effect of endothelium removal on responses to either ET-1 or ET-3 suggests a limited role for relaxant ET_B receptors which may be present on the endothelial cells in these arteries. Similar findings have been reported for larger human coronary arteries *in vitro* (Chester *et al*., 1992; Bax *et al*., 1994). In addition, intracoronary administration of ET-1 in man (Pernow *et al*., 1996) elicited a fall in coronary blood flow without an initial vasodilatation, suggesting the absence of relaxant ET_B receptors in these vessels. Furthermore, a recent study using scanning electron microscopy and radioligand binding demonstrated a lack of [^{125}I]-ET-1 binding to human coronary artery endothelial cells, suggesting a lack of ET receptors (Russell *et al*., 1997).

Does ET receptor subtype expression change with coronary artery diameter?

ET-1 was more potent than ET-3 in arteries from both groups suggesting a predominantly ET_A receptor mediated response. This was confirmed using the novel, non-peptide ET_A receptor antagonist PD156707 (Reynolds *et al*., 1995). PD156707 has high affinity for human native ET_A receptors with selectivity of between 1000 and 14500 for ET_A over ET_B receptors (Maguire *et al*., 1997). PD156707 caused parallel rightward shift of the CRC to ET-1 in both groups, without a change in the maximum response. No portion of the ET-1 curve was resistant to the antagonist, suggesting a predominantly ET_A-induced response. Within individual experiments, in arteries from both groups, lower concentrations of PD156707 would sometimes yield greater shifts than expected. This anomaly has been described previously and may be due to reversion of the sodium salt of the butenolide compound to the less soluble form at higher concentrations (Maguire *et al*., 1997). Alternatively, the non-concentration dependent action of PD156707 in some patients may indicate the presence of more than one ET receptor subtype. Taken together with the biphasic ET-3 response, this may reflect a constrictor ET_B component in some individuals. Nevertheless, the pA_2 values obtained here in coronary arteries of varying internal diameter, are consistent with those described for PD156707 at human ET_A receptors (Maguire *et al*., 1997) and is $\sim$10 fold more potent than that described for animal (rabbit) ET_A receptors (Reynolds *et al*., 1995).

In conclusion, the results indicate that functional ET_A receptors predominate on the smooth muscle layer of human small coronary arteries. There was no evidence for changes in ET receptor subtype expression (e.g. from ET_A to ET_B) along the coronary vascular tree. Visualization of ET receptors on small intramyocardial arteries using autoradiography also demonstrated a predominance of ET_A receptors on the smooth muscle of these vessels. The relative importance of the ET_A receptor subtype in human small coronary artery vasoconstriction suggests that this ET receptor subtype may represent a novel therapeutic target for the treatment of coronary vascular disorders.

This work was supported by grants from the British Heart Foundation, Issac Newton Trust, Royal Society and Medical Research Council. L.N.P. is the recipient of a M.R.C. studentship. We would like to thank the Consultants and theatre staff of Papworth Hospital, Cambridge for permission to collect human tissue. We are grateful to Dr Janet J. Maguire for critical discussion of the manuscript.

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(Received October 13, 1997
Revised February 26, 1998
Accepted March 5, 1998)