British Journal of Pharmacology (1995) 114, 1110–1116 © 1995 Stockton Press All rights reserved 0007–1188/95 $9.00

Endothelin ET_A and ET_B mRNA and receptors expressed by smooth muscle in the human vasculature: majority of the ET_A sub-type

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1 We measured the ratio of ET_A and ET_B sub-types in the media (containing mainly smooth muscle) of human cardiac arteries (aorta, pulmonary and coronary), internal mammary arteries and saphenous veins.

2 In saturation experiments, [^{125}I]-endothelin-1 ([^{125}I]-ET-1) bound with high affinity to the media of each vessel ($n=3$ individuals or homogenate preparations ± s.e.mean):coronary artery, $K_D = 0.14 \pm 0.02$ nM, $B_{max} = 71.0 \pm 21.0$ fmol mg^{-1} protein; pulmonary artery, $K_D = 0.85 \pm 0.25$ nM, $B_{max} = 15.2 \pm 10.3$ fmol mg^{-1} protein; aorta, $K_D = 0.51 \pm 0.02$ nM, $B_{max} = 9.4 \pm 4.4$ fmol mg^{-1} protein; internal mammary artery, $K_D = 0.34 \pm 0.31$ nM, $B_{max} = 2.0 \pm 0.5$ fmol mg^{-1} protein and saphenous vein, $K_D = 0.28 \pm 0.05$ nM, $B_{max} = 52.8 \pm 1.0$ fmol mg^{-1} protein. In each vessel, over the concentration-range tested, Hill slopes were close to unity and a one site fit was preferred to a two site model.

3 In competition binding assays, the ET_A selective ligand, BQ123 inhibited the binding of 0.1 nM [^{125}I]-ET-1 to the media in a biphasic manner. In each case, a two site fit was preferred to a one or three site model: coronary artery, $K_DET_A = 0.85 \pm 0.03$ nM, $K_DET_B = 7.58 \pm 2.27$ μM, ratio = 89:11%; pulmonary artery, $K_DET_A = 0.27 \pm 0.05$ nM, $K_DET_B = 24.60 \pm 5.34$ μM, ratio = 92:8%; aorta, $K_DET_A = 0.80 \pm 0.40$ nM, $K_DET_B = 2.67 \pm 2.60$ μM ratio = 89:11%; saphenous vein, $K_DET_A = 0.55 \pm 0.17$ nM, $K_DET_B = 14.4 \pm 0.26$ μM, 85:15% ($n=3$ individuals or homogenate preparations ± s.e.mean). BQ123 showed up to 18000 fold selectivity for the ET_A over the ET_B sub-type. The ET_A-selective ligand, [^{125}I]-PD151242 labelled 85% of the receptors detected by a fixed concentration of [^{125}I]-ET-1 in media of internal mammary artery, measured by quantitative autoradiography. In contrast, the density of ET_B receptors detected with [^{125}I]-BQ3020 was 7.0 ± 1.5 amol mm^{-2}, representing about 8% of [^{125}I]-ET-1.

4 A single band corresponding to the expected position for mRNA encoding the ET_A receptor (299 base pairs) was found in the media in each of the five vessels ($n=3$ individuals) using reverse-transcriptase polymerase chain reaction assays. A single band corresponding to the ET_B sub-type (428 base pairs) was also always detected.

5 ^{35}S-labelled antisense probes to ET_A and ET_B hybridised to the media of epicardial coronary arteries as well as intramyocardial vessels, confirming the presence of mRNA encoding both sub-types in the vascular smooth muscle of the vessel wall.

6 Although mRNA for both receptors was detected, competition binding using BQ123 demonstrated that the majority (at least 85%) of ET receptors present in smooth muscle are the ET_A sub-type. These results provide further support for the hypothesis that the ET_A sub-type is the receptor that must be blocked in humans to produce a beneficial vasodilatation in pathophysiological conditions where there is an increase in peptide concentration or receptor density.

Keywords: Endothelin; PD151242; BQ123; BQ3020; ET_A mRNA; ET_B mRNA; autoradiography; polymerase chain reaction; aorta; coronary artery; internal mammary artery; pulmonary artery; saphenous vein

Introduction

In man, three genes have been predicted to encode three endothelin (ET) peptides, ET-1, ET-2 and ET-3. Biochemical analysis by radioimmunoassay and high performance liquid chromatography has shown that ET-1 and ET-2 are the most abundant isoforms expressed by the human cardiovascular system (Plumpton *et al.*, 1993). ET-1 and ET-2 (but not ET-3) mRNA has been detected in human endothelial cells (O'Reilly *et al.*, 1993a,b). Immunoreactive mature ET, and the precursors big ET-1 and big ET-2 (but not big ET-3) have been localized to the cytoplasm of endothelial cells from a range of human vascular beds including internal mammary artery and saphenous vein (Howard *et al.*, 1992). The wide-spread distribution of ET-1 (and possibly ET-2) is consistent with a proposed role as a ubiquitous endothelium-derived vasoactive peptide and a corresponding general expression of ET receptors in smooth muscle of the vessel wall would be expected. ET-3 is the only endogenous isoform that is able to distinguish between the two receptor sub-types having low affinity for the ET_A receptor compared to ET-1 but a similar affinity for the ET_B sub-type. Since ET-3 is a poor agonist at ET_A receptors, the predominance of this subtype in the smooth muscle of the human vasculature would be consistent with the lack of expression of ET-3 by endothelial cells of blood vessels.

In support of this hypothesis, in isolated epicardial coronary arteries (Davenport *et al.*, 1993) and renal vessels (Maguire *et al.*, 1994b) ET-1-induced vasoconstriction appears to be mediated mainly by the ET_A sub-type since ET-3 is about two orders of magnitude less potent than ET-1. Secondly, the ET_A-selective antagonists cause a right-ward and parallel shift of the ET-1-induced constriction in these vessels. However, mRNA encoding both sub-types have been detected and binding assays using sub-type selective ligands suggest ET_B as well as ET_A receptors are present.

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In animals, ET-1 can elicit constriction through both ET_A and ET_B receptors although the pattern is complex: the sub-type ratio varies in different vascular beds in the same species and in the same vascular bed in different species (Davenport & Maguire, 1994). For example, constriction appears to be mediated only by ET_A receptors in rat aorta (Sumner *et al.*, 1992) but by ET_B receptors in rabbit pulmonary artery (White *et al.*, 1994a). In porcine coronary artery, the ET_A selective antagonist, BQ123 does not block all of the ET-1-induced contraction, indicating activation of both sub-types (Ihara *et al.*, 1992).

ET-1 is a potent constrictor of human isolated vessels. The peptide has an EC_{50} of 15 nM in aorta (Maguire & Davenport, 1995). In pulmonary arteries, EC_{50} values have a range of 0.1–17 nM (Hay *et al.*, 1993; Fukuroda *et al.*, 1994; Maguire & Davenport, 1995) and in coronary arteries the range is 1–130 nM (Franco-Cereceda, 1989; Davenport *et al.*, 1989b; Godfraind *et al.*, 1993; Maguire & Davenport, 1993; 1994; 1995; Opgaard *et al.*, 1994). In the internal mammary artery, EC_{50} values range from 4–10 nM (Costello *et al.*, 1990; Luscher *et al.*, 1990; Bax *et al.*, 1993; Maguire & Davenport, 1993, 1994; 1995; White *et al.*, 1994b) and 3–18 nM in the sapheneous vein (Luscher *et al.*, 1990; Costello *et al.*, 1990; Maguire & Davenport, 1993; 1994; 1995; Seo *et al.*, 1994; Akar *et al.*, 1994). However, whether the media of all of these vessels express ET_A and ET_B mRNA as well as the corresponding receptor protein has not been examined in detail.

Our aim was to use ligand binding to measure the ratio of ET_A and ET_B sub-types in the media (containing mainly smooth muscle) of human cardiac arteries where animal studies suggest ET-1 constriction occurs only via ET_A (rat aorta), ET_B (rabbit pulmonary artery) or both sub-types (porcine coronary artery). We also compared the internal mammary artery and saphenous veins where ET_B-mediated constrictor responses have been reported (White *et al.*, 1994b; Seo *et al.*, 1994). These results were correlated with the expression of mRNA encoding each receptor using reverse-transcriptase polymerase chain reaction (RT-PCR) assays.

Methods

Vessels

Aorta, pulmonary arteries, left and right main stem coronary arteries were obtained at the time of operation from male and female patients undergoing heart transplantation (age range 43–56 years) for ischaemic heart disease. Saphenous vein and left internal mammary artery were obtained from male and female patients undergoing coronary artery bypass grafts. The endothelial layer was scraped off and the adventitia separated from the media (confirmed by microscopy of haematoxylin and eosin stained sections). Tissue was snap frozen immediately in liquid nitrogen and stored at $-70°C$ until use.

Saturation binding assays: slide mounted sections

For ligand binding in larger vessels (aorta, pulmonary and coronary arteries), sections (10 μm thick) were cut on a cryostat microtome and mounted onto gelatin-coated microscope slides. In saturation experiments, tissue sections were pre-incubated for 15 min in HEPES buffer as previously described (Davenport *et al.*, 1989a,b; 1991; Karet & Davenport, 1993). Briefly, sections were then incubated with increasing concentrations (8 pM–8 nM) of [^{125}I]-ET-1 in incubation buffer for 2 h at 23°C. Non-specific binding was defined with 1 μM of unlabelled ET-1. Sections were rinsed in Tris-HCl buffer (0.05 M, pH 7.4) at 4°C (3 × 5 min) and the amount of radioactivity measured in a gamma counter.

Homogenate binding

Frozen saphenous veins and internal mammary arteries (typically from a minimum of 30 individuals to obtain sufficient tissue) were homogenized in ice cold 50 mM Tris-HCl buffer, pH 7.4 (containing 5 mM $MgCl_2$, 5 mM EDTA, 1 mM EGTA, 10,000 units ml^{-1} aprotinin). The homogenate was centrifuged at 1000 *g* for 1 min at 4°C, the pellet discarded and the supernatant re-centrifuged at 40,000 *g* for 30 min. The resulting pellet was washed and resuspended three times in the Tris-HCl buffer. The final pellet was resuspended in 50 mM HEPES buffer, pH 7.4 (containing 5 mM $MgCl_2$, 0.3% wt/vol bovine serum albumen). After measuring the protein, the membranes were diluted to give typical concentrations of about 6 mg ml^{-1} protein.

Aliquots (100 μl) were added to tubes containing increasing amounts of [^{125}I]-ET-1 (8 pM–8 nM) in HEPES incubation buffer for 2 h at 23°C. Non-specific binding was determined with 1 μM unlabelled ET-1. The final protein concentration was 2 mg ml^{-1}. Each assay was terminated by centrifugation (20,000 *g*, 10 min, 4°C) to separate bound from free radioligand. The supernatants were discarded and the pellets resuspended in 1 ml ice cold 50 mM Tris-HCl buffer (pH 7.4 at 4°C). The tubes were re-centrifuged (20,000 *g*, 10 min, 4°C), the supernatants discarded and the pellets counted in a gamma counter.

Competition experiments

Competition assays were carried out under the conditions described above. Sections of aorta, pulmonary and coronary artery or homogenate of saphenous vein were incubated with 0.1 pM [^{125}I]-ET-1 and increasing concentrations (20 pM–100 μM) of BQ123. Non-specific binding was defined by use of 1 μM unlabelled ET-1. The results of binding experiments were analysed with EBDA and LIGAND programmes as previously described (Molenaar *et al.*, 1992; 1993; Davenport *et al.*, 1994a). The presence of 1, 2 or 3 sites was tested by the *F*-ratio test in LIGAND. The model adopted was that which provided the best fit ($P<0.05$).

Autoradiography

Quantitative autoradiography was used to compare the density of ET_A and ET_B receptors in the internal mammary artery, as there was insufficient material for competition binding assays. Sections of internal mammary artery (as well as aorta, pulmonary, coronary arteries and saphenous veins) from three individuals were incubated in buffer containing either 0.1 nM [^{125}I]-ET-1 to visualize all ET receptors, 0.1 nM [^{125}I]-PD151242 to visualize ET_A (Davenport *et al.*, 1994a,c; Peter & Davenport, 1994; 1995) or 0.1 nM [^{125}I]-BQ3020 (Molenaar *et al.*, 1992; 1993) to detect the ET_B sub-type. [^{125}I]-PD151242 has previously been shown to be highly selective for the human ET_A sub-type and the unlabelled compound is a competitive antagonist causing a parallel rightward shift of the ET-1 concentration-response curve in human isolated coronary arteries (Davenport *et al.*, 1994a). [^{125}I]-BQ3020 is selective for the human ET_B sub-type (Molenaar *et al.*, 1992; 1993) and a potent agonist at animal ET_B receptors (Gardiner *et al.*, 1994). In each case, non-specific binding was determined by incubating adjacent consecutive sections in the presence of 1 μM of the corresponding unlabelled peptides. Sections were exposed to radiation-sensitive film (Hyperfilm βmax) with radioactive standards and quantified by computer-assisted densitometry as previously described (Davenport *et al.*, 1988).

Reverse-transcriptase polymerase chain reaction (RT-PCR) assays

Total RNA was extracted from the media of aorta, pulmonary, coronary, internal mammary arteries and saphenous

veins from three individuals by a single step guanidinium isothiocyanate method. cDNA synthesis and RT-PCR assays were carried out as previously described (O'Reilly *et al.*, 1992; 1993a,b; Karet *et al.*, 1994). Avian myoblastosis virus reverse transcriptase was used to synthesize first strand cDNA from 2.5 μg RNA. Nested PCR amplification was carried out using 1 μl cDNA template, 5 μl reaction buffer (100 nM Tris-HCl, pH 8.3 at 25°C, 500 mM KCl and 15 mM $MgCl_2$), 5 μl dNTPs (2 mM), 5 μl of each of the appropriate oligonucleotide primer pairs (designed from published nucleotide sequences) and 2.5 U *Taq* DNA polymerase. PCR products were separated by agarose gel electrophoresis and the bands stained with ethidium bromide. These previously cloned and sequenced PCR products show 100% homology with published sequences for ET_A or ET_B (O'Reilly *et al.*, 1992).

In situ *hybridization*

The distribution of ET_A and ET_B mRNA was compared in more detail in coronary arteries by *in situ* hybridization. Assays were carried out as previously described (Molenaar *et al.*, 1993). Briefly, ET_A (bases 439–737) and ET_B (bases 497–924) PCR amplified DNA bands were cloned into pBluescript II KS (Stratagene Inc., La Jolla, California, U.S.A.) and the linearised inserts used as a template to generate ^{35}S labelled sense and antisense RNA probes. Sections of epicardial coronary artery and intra-ventricular septum containing intra-myocardial vessels were exposed to radiation-sensitive film together with standards. The amount of probe specifically hybridized was determined by digitally subtracting the autoradiographical image of the sense control probe from that of the antisense probe by computer-assisted densitometry as previously described (Davenport & Nunez, 1990).

Table 1 Dissociation constants (K_D), maximal density of receptors (B_{max}) and Hill coefficients (n_H) for [^{125}I]-endothelin-1 ([^{125}I]-ET-1) binding in human vessels

	K_D (nM)	B_{max} (fmol mg^{-1} protein)	n_H
Arteries:			
Coronary	0.14 ± 0.02	71.0 ± 21.0	0.99 ± 0.01
Pulmonary	0.85 ± 0.25	15.2 ± 10.3	0.85 ± 0.08
Aorta	0.51 ± 0.02	9.4 ± 4.4	0.85 ± 0.11
Internal mammary	0.34 ± 0.31	2.0 ± 0.5	0.92 ± 0.10
Vein:			
Saphenous	0.28 ± 0.05	52.8 ± 1.0	0.89 ± 0.01

For aorta, coronary and pulmonary arteries values are the mean ± s.e.mean of three individuals; for saphenous vein and internal mammary artery values are the mean ± s.e.mean of three separate experiments using vessels pooled from about 30 individuals.

Drugs

BQ123, (cyclo [D-Asp-L-Pro-D-Val-L-Leu-D-Trp-], Ihara *et al.*, 1992) and BQ3020, [Ala^{11,15}]Ac-ET-1$_{(6-21)}$ (Molenaar *et al.*, 1992) were synthesized by solid phase t-Boc chemistry. PD151242, (N-[(hexahydro-1-azepinyl)carbonyl]L-Leu(1-Me) D-Trp-D-Tyr (Davenport *et al.*, 1994a,c) was synthesized and supplied by Dr A.M. Doherty (Parke-Davis Pharmaceutical Research Division). Peptide concentrations were determined by u.v. spectrophotometry. [^{125}I]-ET-1, [^{125}I]-PD151242 and [^{125}I]-BQ3020 were from Amersham International plc, (Amersham, UK); unlabelled ET-1 from Novabiochem (Nottingham, UK). Taq polymerase was from Perkin-Elmer Cetus (London, UK), reverse-transcriptase from Anglian Biotech (Essex, UK). All other reagents were from Sigma Chemical Co. (Poole, Dorset, UK), BDH (Poole, Dorset, UK) or Fisons (Loughborough, Leicester, UK).

Results

Ligand binding

[^{125}I]-ET-1 bound with a similar sub-nanomolar affinity to the media of all of the vessels examined (Table 1). In each vessel, over the concentration-range tested, Hill slopes were close to unity and a one site fit was preferred to a two site model. The highest densities of ET receptors were present in the coronary arteries; lowest levels were detected in the internal mammary artery.

BQ123 competed for the binding of a fixed concentration of [^{125}I]-ET-1 in a biphasic manner in the media of all vessels (Table 2, Figure 1). In each case, a one site model could not be fitted to these data and a two site fit was preferred to a three site model, giving a high nanomolar affinity corresponding to the ET_A receptor and a low, micromolar site corresponding to the ET_B receptor. BQ123 showed up to 18 000 fold selectivity for the ET_A over the ET_B sub-type. In each case ET_A receptors predominated, with the ET_B subtype representing less than 15% of the ET receptors in smooth muscle cells of the human vasculature.

Autoradiography

Owing to the small size of internal mammary arteries obtained following surgery it was not possible to obtain sufficient receptor protein to carry out competition assays and relative densities were compared by quantitative autoradiography. The density of ET_A binding in the media of internal mammary artery following incubation with a fixed concentration of [^{125}I]-PD151242 was 78.7 ± 5.9 amol mm^{-2}, representing about 85% of the [^{125}I]-ET-1 binding (92.9 ± 4.6 amol mm^{-2}). In contrast, the density of ET_B receptors detected with [^{125}I]-BQ3020 was 7.0 ± 1.5 amol mm^{-2}, representing about 8% of [^{125}I]-ET-1. As expected from the competition binding assays, a similar pattern was observed in the media from aorta, pulmonary and coronary arteries as well

Table 2 Inhibition of [^{125}I]-endothelin-1 ([^{125}I]-ET-1) binding to media from human vessels by the ET_A selective BQ123

	K_D ET_A (nM)	ET_B (μM)	B_{max} ET_A (fmol mg^{-1}protein)	ET_B	Ratio ET_A:ET_B
Arteries:					
Coronary	0.85 ± 0.03	7.58 ± 2.27	15.4 ± 4.1	1.7 ± 0.5	89:11
Pulmonary	0.27 ± 0.05	24.60 ± 5.34	27.2 ± 3.0	2.4 ± 0.6	92:8
Aorta	0.80 ± 0.40	2.67 ± 2.60	9.4 ± 1.7	1.1 ± 0.4	89:11
Vein:					
Saphenous	0.55 ± 0.17	14.4 ± 0.26	35.3 ± 20.2	6.5 ± 1.5	85:15

Values are mean ± s.e.mean of 3 individuals.

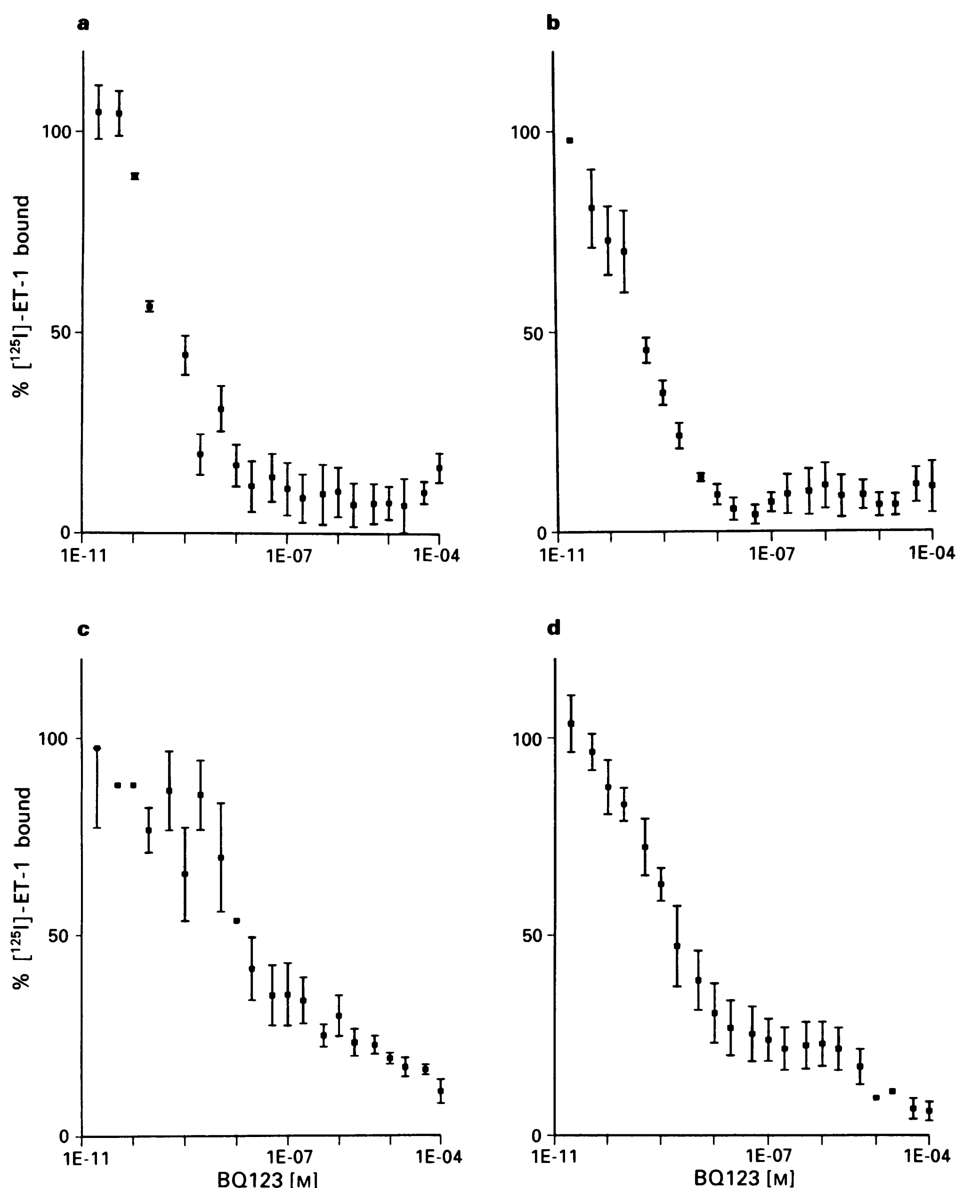

Figure 1 Inhibition of 0.1 nM [^{125}I]-endothelin-1 ([^{125}I]-ET-1) binding by BQ123 to slide mounted sections of main stem aorta (a), pulmonary artery (b), coronary artery (c) and saphenous vein (d). In each case, with the exception of the aorta, over the concentration-range tested, BQ123 competed in a biphasic manner and a two site fit was preferred to a one site or three site model. (Each value represents the mean ± s.e.mean of three individuals except for saphenous vein where homogenates were prepared from about 30 vessels).

as the saphenous vein, with the density of ET_B receptors being much lower in the smooth muscle than densities of the ET_A sub-type localized by [^{125}I]-PD151242.

Molecular biology

Agarose gels showing the presence of PCR products of the expected size corresponding to mRNA encoding both the ET_A receptor (299 base pairs) and ET_B receptor (428 bases pairs) were detected in the media obtained from three individuals in each of the four arteries and saphenous vein. Bands were not detected in the negative control lane in which the template had been omitted (Figure 2).

In situ *hybridization*

The ^{35}S-labelled antisense ET_A probe hybridized to the media of epicardial coronary arteries as well as intramyocardial vessels. The autoradiographic image of the sense control probe was digitally subtracted from that of the antisense probe to give the amount specifically hybridized of 90 ± 8 amol mm^{-2} in epicardial coronary arteries and 50.0 ± 3 amol mm^{-2} in intramyocardial vessels (mean ± s.e.mean of at least 3 vessels). The amount of non-specific hybridization detected by the sense control probe was higher for the ET_B receptor but specific hybridization could still be detected, 34.3 ± 3 amol mm^{-2} and 43.0 ± 8 amol mm^{-2} in epicardial and intra-myocardial vessels respectively.

Discussion

[^{125}I]-ET-1 bound with a similar sub-nanomolar affinity to the media containing mainly vascular smooth muscle, in all of the human blood vessels examined. Over the concentration-range tested, Hill slopes were close to unity and a one site fit was preferred to a two site model. These results suggest the presence of either a single population of receptors or a

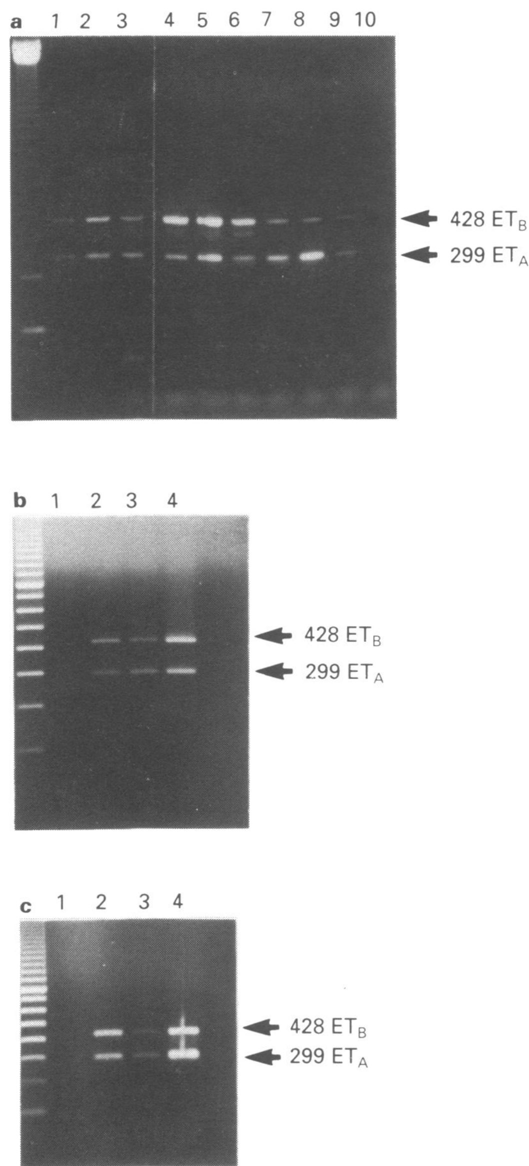

Figure 2 Detection of ET_A and ET_B mRNA in human vessels. Agarose gel electrophoresis of reverse-transcriptase polymerase chain reaction (RT-PCR) products from the media (containing mainly vascular smooth muscle cells) of vessels obtained from different vascular beds. The presence of PCR bands corresponding to size predicted for ET_A (299 base pairs) and ET_B (428 base pairs) mRNA was detected in the media of all vessels examined. (a) Lane 1–3, aorta from three individuals; lane 4–6, pulmonary artery from three individuals; lane 7–9, coronary artery from three individuals. Lane 10, negative control where cDNA template had been omitted. The marker is a 123-base pair ladder. (b) Lane 1, negative control where cDNA template had been omitted. Lane 2–4, saphenous vein from three individuals. The marker is a 100-base pair ladder. (c) Lane 1, negative control where cDNA template had been omitted. Lane 2–4, internal mammary artery from three individuals. The marker is a 100-base pair ladder.

heterogeneous population with the same affinity for the peptide. In the majority of human tissues examined using homogenates or slide mounted sections, labelled ET-1 binds monophasically with affinities ranging from about 0.01–10 nM, with receptor densities of about 10 to 10 000 fmol mg^{-1} protein (Davenport *et al.*, 1994d). The range of K_D values in the media of these five vessels (0.14–0.85 nM) compare with values estimated by the same technique in other types of human muscle. For example, in human myometrium (consisting of mainly non-vascular smooth muscle), K_D values were 1.2 nM (Bacon *et al.*, 1993). In cardiac muscle, K_D values of 0.60 and 0.35 nM were obtained in atria and ventricle (Molenaar *et al.*, 1993). The range of affinities for [^{125}I]-ET-1 binding to homogenates of placental arteries and veins (presumed to contain media, adventitia and endothelium) was 0.026–0.045 nM (Robaut *et al.*, 1991). Thus [^{125}I]-ET-1 binds with high affinity in the media of these vessels although the density of binding is comparatively low compared to other human tissue. The affinities calculated by ligand binding for the five vessels show a positive correlation with the EC_{50} values for the contractile effects of this peptide on the isolated vessels denuded of endothelium (Maguire & Davenport, 1995).

A consistent pattern emerged with the detection in all vessels of receptor mRNA using RT-PCR. A single PCR product corresponding to the expected position for ET_A mRNA was detected in media of each of these vessels. A single band corresponding to ET_B mRNA was also always found. Localization of ET_B as well as ET_A mRNA to vascular smooth muscle cells was confirmed by *in situ* hybridization in sections of coronary arteries. Single bands corresponding to ET_A and ET_B mRNA have also been found in renal veins and arteries by RT-PCR (Maguire *et al.*, 1994b) and by Northern analysis in internal mammary artery (Seo *et al.*, 1994). Expression of mRNA encoding both sub-types has been found in a range of human tissues including ventricle (Molenaar *et al.*, 1993), endometrium (O'Reilly *et al.*, 1992) and kidney (Davenport *et al.*, 1993b). In these studies, detection of mRNA correlates with expression of receptor protein. Using the same RT-PCR assay conditions with isolated cells, only ET_B mRNA was detected in human vascular endothelial cells whereas rat aortic smooth muscle cells express ET_A mRNA (Molenaar *et al.*, 1993) and only the ET_A receptor protein could be detected (Davenport *et al.*, 1994b). However, in isolated myocytes mRNA encoding both sub-types was found and BQ123 inhibited binding of iodinated ET-1 biphasically, confirming that both receptor sub-types were present in these cells (Molenaar *et al.*, 1993).

The ET_A selective ligand, BQ123, inhibited [^{125}I]-ET-1 binding in a biphasic manner in both arteries (aorta, pulmonary and coronary arteries) as well as saphenous vein in agreement with the molecular biology studies. Quantitative autoradiography showed that while a small population of ET_B receptors could be detected in the internal mammary artery, the ET_A sub-type was again more abundant. Similar results have been obtained with other human vessels. In human kidney, ET_A receptors detected with [^{125}I]-PD151242 were mainly localized to the vasculature and micro-autoradiography demonstrated smaller vessels such as arterioles expressed the ET_A sub-type (Karet *et al.*, 1993).

BQ123 competes for [^{125}I]-ET-1 binding for an ET_A site in the nanomolar range and ET_B site at micromolar concentrations in a number of human tissues including cardiac muscle (Molenaar *et al.*, 1993), myometrium (Bacon *et al.*, 1993) and kidney cortex and medulla (Karet *et al.*, 1993). Intriguingly, Bax *et al.* (1993) found while BQ123 competed in a biphasic manner for [^{125}I]-ET-1 binding in human cardiac muscle the compound detected only one site in coronary arteries. Two sites were detected when BQ123 competed for [^{125}I]-sarafotoxin S6b in this artery. This was interpreted as evidence of a non-ET_A/ET_B site. The RT-PCR assays used in this study have not revealed the presence of additional bands. In competition binding assys, BQ123 competed with similar affinities in coronary artery as all other vessels where Schild slopes of unity have also been found (Maguire & Davenport, 1995).

These studies show that human vessels express a small population of ET_B receptors. In animals, a complex pattern of ET-1-induced vasoconstriction has emerged, with a significant ET_B component in some vessels (see for example, Davis *et al.*, 1991; Clozel *et al.*, 1992; Sumner *et al.*, 1992; Cristol *et al.*, 1993; Gardiner *et al.*, 1994; Pollock & Opgenorth, 1993; 1994; Wellings *et al.*, 1994; White *et al.*, 1994a) whereas constriction appears to be mediated only by ET_A receptors in rat aorta (Sumner *et al.*, 1992) or rabbit

renal artery (Telemarque *et al.*, 1993) but both sub-types are activated in porcine coronary artery (Ihara *et al.*, 1992).

Although the ET_B agonists BQ3020 and [Ala^{1,3,11,15}]-ET-1 are potent constrictors of some rat vascular beds (Bigaud & Pelton, 1992; Gardiner *et al.*, 1994), they had no detectable action on the isolated vessels used in this study at concentrations up to 3 μM (Davenport *et al.*, 1993; Maguire & Davenport, 1995). The non-endogenous ET_B agonist in mammals, sarafotoxin S6c, was also inactive in aorta and pulmonary artery (Maguire & Davenport, 1995). This peptide does cause vasoconstriction in some human vessels such as saphenous vein (White *et al.*, 1994b, Maguire & Davenport, 1995), internal mammary artery (Seo *et al.*, 1994; Maguire & Davenport, 1995) and vein (Seo *et al.*, 1994), omental arteries and veins (Riezebos *et al.*, 1994) and pulmonary arteries (Hay *et al.*, 1993; Maguire & Davenport, 1995). Where responses did occur, these can be variable, for example a response was detected in saphenous vein in only about 50% (White *et al.*, 1994) of individuals or less (Maguire & Davenport, 1995). Although the constrictor actions of this snake venom are potent, the magnitude of the response is much less than that of ET-1. Therefore whilst some individuals may have functional constrictor ET_B receptors, the poor response to ET-3 in these vessels (Maguire & Davenport, 1994, Davenport & Maguire, 1995) would suggest that these receptors may have limited physiological importance in the cardiovascular system.

Ligand binding has demonstrated that the majority (at least 85%) of ET receptors present in smooth muscle from the five vessels are of the ET_A sub-type. Functional studies have shown that ET_A-selective antagonists in these vessels cause a parallel, rightward shift of the ET-1 concentration-response curve and Schild slopes were not significantly different from unity (Maguire & Davenport, 1995; Maguire *et al.*, 1994a,c). This contrasts with porcine coronary artery, where a portion of the ET-1 response mediated via presumed ET_B receptors is not blocked by BQ123 (Ihara *et al.*, 1992).

In other human vessels, ET-1 is always more potent, with the ET-3 dose-response curve shifted to the right. ET-3 is at least 100 fold less potent that ET-1 in omental veins (Riezebos *et al.*, 1994), renal arteries and veins (Maguire *et al.*, 1994b), umbilical artery (Bodelsson & Stjernquist, 1993) or inactive in omental arteries (Riezebos *et al.*, 1994). Where full concentration-response curves have been carried out comparing ET-1 and ET-3, human vessels have not yet been identified in which ET-3 is equipotent to ET-1 as in the rabbit pulmonary artery (White *et al.*, 1994a).

In the human vasculature, a consistent pattern is emerging: although both receptors can be detected by ligand binding, *in vitro* pharmacological experiments suggest that vasoconstriction in man is mediated predominantly via the ET_A sub-type. Taken together, these results provide further support for the hypothesis that the ET_A sub-type is the receptor that must be blocked in human patients to produce a beneficial vasodilatation in pathophysiological conditions where there are elevated concentrations of the peptide or increases in receptor density. ET_A selective drugs may have an additional advantage in avoiding blockade of the ET_B sub-type present on endothelial cells since activation of these receptors by ET-1 is thought to release endothelium-derived relaxing factors.

We thank the surgeons and theatre staff of Papworth Hospital for their co-operation in obtaining tissue and Dr A.M. Doherty (Parke-Davis Pharmaceutical Research Division) for synthesizing and supplying PD151242. Supported by grants from the British Heart Foundation, Royal Society, BBSRC, Isaac Newton Trust.

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(Received August 3, 1994
Revised November 9, 1994
Accepted November 10, 1994)