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Endothelin receptors in human coronary artery and aorta

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1 ET_A and ET_B-selective and non-selective ligands were used to define the endothelin receptors in the media (vascular smooth muscle layer) of human aorta and coronary artery. Saturation experiments with iodinated endothelin-1 (ET-1), endothelin-2 and sarafotoxin 6b (S6b) identified high affinity binding sites in aorta (K_D [^{125}I]-ET-1 0.33±0.02 nM (n=9), K_D [^{125}I]-ET-2 1.04±0.23 nM (n=5), K_D [^{125}I]-S6b 0.15±0.01 nM (n=9 ±s.e.mean)) and coronary artery (K_D [^{125}I]-ET-1 0.43±0.10 nM, K_D [^{125}I]-ET-2 0.71±0.17 nM, K_D [^{125}I]-S6b 0.27±0.03 nM (n=3 ±s.e.mean)). Hill coefficients (n_H) approached unity in each case.

2 No specific binding was detectable with [^{125}I]-ET-3 (4 pM-4 nM) in aorta. Unlabelled ET-3 competed monophasically with [^{125}I]-ET-1 in aorta (K_D, 8.21±1.62 nM, compared to unlabelled ET-1 K_D, 0.60±0.20 nM) (n=3 ±s.e.mean). In coronary artery, the K_D and B_{max} values calculated from [^{125}I]-ET-3 saturation experiments were 2.13±1.39 nM and 20.6±12.9 fmol mg^{-1} protein, respectively (n=3 ±s.e.mean).

3 ET_A antagonists competed monophasically for [^{125}I]-ET-1 (100 pM) binding sites with nanomolar or subnanomolar affinity in the aorta (K_D BQ123, 0.47±0.13 nM; K_D FR139317, 0.40±0.10 nM; K_D PD151242, 2.09±0.48 nM) and coronary artery (K_D FR139317, 0.41±0.13 nM; K_D PD151242, 3.60±0.74 nM) (n=3 ±s.e.mean). However, two site fits were preferred on analysis of competition experiments with ET_B-selective agonists versus [^{125}I]-ET-1 in coronary artery (BQ3020: $K_D ET_A$ 0.96±0.14 μM, K_D ET_B 1.34±1.08 nM and sarafotoxin 6c: K_D ET_A 1.15±0.14 μM, K_D ET_B 1.77±0.72 nM) (n=3 ±s.e.mean). The selectivity of the agonists for ET_B receptors (700 fold) was lower than reported in other species.

4 Sarafotoxin 6b (2 pM-2 μM) completely inhibited [^{125}I]-ET-1 (100 pM) binding in aorta (K_D 1.36±0.22 nM) (n=3 ±s.e.mean). The non-peptide compounds Ro462005 and bosentan, competed with [^{125}I]-ET-1 binding in coronary artery with K_D values of 0.19±0.04 μM and 2.94±0.95 nM, respectively (n=3 ±s.e.mean).

5 Inhibition of [^{125}I]-ET-2 and [^{125}I]-S6b binding by FR139317 was similar to the inhibition of [^{125}I]-ET-1 binding in both arteries, being monophasic with K_D values in the same range.

6 ET_A receptors in coronary artery media were detected by [^{125}I]-PD151242 (K_D 0.23±0.04 nM, B_{max} 10.1±1.2 fmol mg^{-1} protein) (n=3 ±s.e.mean). [^{125}I]-BQ3020, an ET_B-selective radioligand, indicated the presence of a smaller population of ET_B receptors in this tissue (K_D 0.60±0.31 nM, B_{max} 4.5±2.1 fmol mg^{-1} protein) (n=3 ±s.e.mean).

7 Autoradiography with [^{125}I]-PD151242 and [^{125}I]-BQ3020 confirmed the predominance of ET_A receptors in the media of both arteries.

8 The results of this study indicate that ET_A receptors predominate in the vascular smooth muscle of human cardiac arteries, with a small and variable population of ET_B receptors detectable in the coronary artery.

Keywords: Endothelin receptors; ET_A receptors; ET_B receptors; sarafotoxin; human coronary artery; human aorta; vascular smooth muscle

Introduction

Of the receptors interacting with the potent vasoconstrictor endothelin (ET), two human subtypes have been cloned, the ET_A (Sakurai *et al.*, 1992) and the ET_B (Sakurai *et al.*, 1990; Sakamoto *et al.*, 1991). The ET_A subtype is believed to have higher affinity for ET-1, ET-2 and sarafotoxin 6b (S6b) compared to ET-3 whereas ET_B has equal affinity for the ET-1, ET-2 and ET-3 isopeptides and S6b (Kloog & Sokolovsky, 1989; Sokolovsky, 1994; Watson & Girdlestone, 1995). Vasoconstriction is stimulated by the interaction of endothelin with ET_A receptors on vascular smooth muscle (Sakurai *et al.*, 1992). Whilst contractile ET_B receptors may also be present in the smooth muscle, endothelial ET_B receptors give rise to vasodilatation via the release of substances such as prostacyclin and nitric oxide (De Nucci *et al.*, 1988).

In blood vessels, endothelin is thought to be released predominantly from the abluminal aspect of the endothelium and to act in a paracrine manner on the neighbouring smooth muscle (Wagner *et al.*, 1992). However, in a number of cardiovascular disorders elevated levels of circulating endothelin have been detected (Lerman *et al.*, 1991; Stewart, 1993; Wei *et al.*, 1994). Additionally a role for endothelin has been suggested in experimental models of vasospasm (Clozel *et al.*, 1993). Thus endothelin receptor antagonists may have therapeutic potential in conditions characterized by endothelin-mediated vasoconstriction. Clearly it is important to delineate which receptors are involved in vasoconstriction in man.

Complicating this, there are marked species differences in tissue distribution and apparently in function of the receptor subtypes. In rats for example, the renal vasculature expresses contractile ET_B receptors (Cristol *et al.*, 1993; Wellings *et al.*, 1994) whilst human renal vessels express mainly the ET_A subtype (Karet *et al.*, 1993; Maguire *et al.*, 1994b). ET_B-mediated constriction has been demonstrated in vascular smooth muscle from a variety of sources including rabbit jugular vein (Sumner *et al.*, 1992) and in the rat *in vivo* (Gardiner *et al.*, 1994). A significant contractile ET_B component has also

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been described in porcine coronary artery (Harrison *et al.*, 1992). In contrast, although mRNA encoding both ET_A and ET_B receptors has been detected in the media of human vessels such as coronary artery (Davenport *et al.*, 1993; 1995), only small and inconsistent constrictions to ET_B agonists and ET-3 have been observed with these vessels *in vitro* (Davenport & Maguire, 1994; Opgaard *et al.*, 1994; Maguire & Davenport, 1995).

The aim of this study was to characterize the endothelin receptor population in the media of human coronary artery, an example of a muscular artery and aorta, an elastic artery. As an initial step, a comparison was made of the affinities of iodinated ET-1, ET-2, ET-3 and sarafotoxin 6b, the peptides currently used to classify endothelin receptors. Subtype selective ligands were then used to confirm this initial classification and to seek evidence for the expression of ET_B receptors in human vascular smooth muscle. Previous studies have suggested that ET-1 and S6b may act through distinct, atypical receptors which differ in sensitivity to antagonism by ET_A antagonists (Bax *et al.*, 1993a, b; Bodelsson & Stjernquist, 1993; Maguire *et al.*, 1995). In order to investigate the possible existence of additional subtypes, ET_A-selective ligands were tested in competition with [^{125}I]-ET-1 or [^{125}I]-S6b binding to examine whether the resulting competition curves were monophasic or biphasic.

Drugs

ET-1, ET-2, ET-3, S6b and S6c were from Novabiochem (Nottingham, U.K.) BQ123 (Cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]) and BQ3020 ([Ala$^{11, 15}$]Ac-ET-$1_{(6\text{-}21)}$) were synthesized by solid phase t-Boc chemistry. Peptide concentrations were determined by u.v. spectrophotometry. FR139317 ((**R**)2-[(**R**)-2-[(**S**)-2-[[1-(hexahydro-1H-azepinyl)]-carbonyl]amino-4-methyl-pentanoyl] amino-3- [3-(1-methyl-1H-indolyl)]-propionyl]amino-3-)2-pyridyl)propionic acid), PD151242 (N-[(hexahydro-1-azepinyl) carbonyl]L-Leu(1-Me)D-Trp-D-Tyr), Ro462005 (4-*tert*-butyl-N-[6-(2-hydroxy-ethoxy)- 5-(3-methoxy-phenoxy)-4-pyrimidinyl]-benzenesulphonamide) and bosentan (4-*tert*-butyl-N-[6-(2-hydroxy -ethoxy)-5-(2-methoxy - phenoxy) -2,2′- bipyrimidin-4-yl]-benzenesulphonamide) were synthesized by Dr A. M. Doherty (Parke-Davis Pharmaceutical Research Division). [^{125}I]-ET-1, [^{125}I]-ET-2, [^{125}I]-ET-3, [^{125}I]-S6b, [^{125}I]-PD151242 and [^{125}I]-BQ3020 (1800–2000 Ci mmol^{-1}) were from Amersham International plc (Amersham, U.K.).

Methods

Tissue samples

Samples of human coronary arteries were obtained from 23 male and female patients undergoing cardiac transplantation for ischaemic heart disease, cardiomyopathy or congenital heart disease. Drug therapy included calcium antagonists, vasodilators, angiotensin-converting enzyme inhibitors, diuretics, digoxin and anticoagulants. The average age of these patients was 49 ± 11 years (mean ± s.e.mean). Sixteen different samples of aorta were obtained from patients undergoing transplantation for pulmonary disease or from tissue not used for transplantation. Endothelial cells were removed from each artery by gentle rubbing and the adventitia separated from the media. The remaining smooth muscle layer of the media was snap frozen in liquid nitrogen to be stored at −70°C. Cryostat sections were cut to a 10 μm thickness and thaw-mounted on gelatinised slides.

Saturation binding experiments

Slide-mounted sections were preincubated for 15 min in buffer (50 mM HEPES, 5 mM $MgCl_2$, 0.3% bovine serum albumin, pH 7.4). For saturation binding assays, adjacent sections were subsequently incubated for 2 h at 22°C with HEPES buffer containing radioligand (4 pM–4 nM) as previously described (Molenaar *et al.*, 1993). Non-specific binding was assessed at each radioligand concentration by incubation with the corresponding unlabelled ligand (1 μM). The larger amount of aortic tissue available permitted a more detailed comparison of ET-1 and S6b binding in aorta. Saturation experiments with [^{125}I]-ET-1 and [^{125}I]-S6b were performed concurrently with sections of aorta taken from the same nine individuals.

Competition binding experiments

Tissue sections were incubated with [^{125}I]-ET-1 (100 pM), [^{125}I]-ET-2 (300 pM) or [^{125}I]-S6b (100 pM) in order to label similar proportions of receptors. Unlabelled competing ligands, typically in the range 2 pM–2 μM, were included in the competition binding assays. Non-specific binding was defined in the presence of 1 μM unlabelled ET-1, ET-2 or S6b and total binding in the absence of competing ligand.

Washing in three volumes of 0.05 M Tris-HCl buffer (pH 7.4, 4°C) for 15 min removed unbound ligand. Sections were wiped onto filter papers and bound radioactivity determined by gamma counting. To determine protein content, sections were solubilized in 0.5 M NaOH, 1% sodium dodecyl sulphate for 30 min at 80°C and assayed by the Bio-Rad DC 96-well microtitre plate method (Bio-Rad Laboratories, Herts, U.K.).

Autoradiography

Autoradiography was performed with transverse sections of vessels incubated with (100 pM) [^{125}I]-ET-1, [^{125}I]-PD151242 or [^{125}I]-BQ3020 in order to label 14–30% of receptors. Sections were incubated in the absence or presence of unlabelled peptide (1 μM) under the conditions described above. After washing, the sections were rinsed in deionized water, dried and apposed to Hyperfilm βmax radiation-sensitive film (Amersham International, Amersham, U.K.) for seven days at 22°C.

Statistical analysis

The iterative non-linear curve fitting programme LIGAND was used to analyse binding data (Munson & Rodbard, 1980; McPherson, 1985). The presence of 1, 2 or 3 sites was tested by the *F*-ratio test and the model adopted was that which provided the best fit ($P<0.05$). Significance of difference between binding parameters was tested by the Mann Whitney U test ($P<0.05$). The larger sample size ($n=9$) used in the comparison of [^{125}I]-ET-1 and [^{125}I]-S6b B_{max} values permitted the use of the Student's *t* test ($P<0.05$).

Results

Saturation experiments

Iodinated ET-1, ET-2 and S6b bound with similar high affinity to the media of human aorta. Hill slopes approached unity (Table 1). In contrast, no specific binding was demonstrated with [^{125}I]-ET-3 or the ET_B-selective radioligand, [^{125}I]-BQ3020. In coronary artery, iodinated ET-1, ET-2 and S6b also bound with subnanomolar affinity and Hill slopes close to unity. A one site fit was obtained with [^{125}I]-ET-3 in coronary artery (Table 2). Saturation experiments with an ET_A-selective radioligand, [^{125}I]-PD151242 (Davenport *et al.*, 1994a, b), detected ET_A receptors in all the individuals tested (B_{max} 10.1 ± 1.2 fmol mg^{-1} protein). Experiments with [^{125}I]-BQ3020 also revealed the presence of a small population of ET_B receptors in coronary artery media (Table 2).

Competition experiments

The ET_A-selective antagonists, BQ123, FR139317 and PD151242 competed monophasically with nanomolar or subnanomolar affinity for the binding of [^{125}I]-ET-1 in aortic

media (Table 3). One site models were preferreed to two or three site fits on LIGAND analysis of these data. Unlabelled ET-3 also competed monophasically in aorta with 14 fold lower affinity than unlabelled ET-1, consistent with binding at ET_A receptors. In agreement, the ET_B-selective agonist, S6c was a weak competitor, only competing at concentrations greater than 1 nM with 50% specific [^{125}I]-ET-1 binding remaining at 1 μM indicating binding with lower affinity to ET_A sites.

In coronary artery, two sites were detectable in competition experiments with ET_B-selective agonists. BQ3020 inhibited [^{125}I]-ET-1 binding in coronary artery biphasically (K_D ET_B 1.34±1.08 nM, B_{max} 11.9±1.2 fmol mg^{-1} protein; K_D ET_A 0.96±0.14 μM, B_{max} 23.1±1.2 fmol mg^{-1} protein) (n=3, mean±s.e.mean). In two out of five coronary arteries tested S6c competed very poorly for ET-1 binding sites. In the remaining three vessels, high affinity sites (K_D ET_B 1.77±0.72 nM, B_{max} 7.9±1.1 fmol mg^{-1} protein) as well as low affinity sites (K_D ET_A 1.15±0.14 μM, B_{max} 8.9±1.2 fmol mg^{-1} protein) (n=3, mean±s.e.mean) were detectable. Thus these agonists appear to have much lower selectivity for human ET_B receptors than previously reported in tissues from other species such as the pig and rat (Williams *et al.*, 1991; Ihara *et al.*, 1992).

Table 1 Dissociation constants (K_D), maximal density of receptors (B_{max}) and Hill coefficients (n_H) derived from saturation binding experiments with the media of human aorta

Radioligand	n	K_D (nM)	B_{max} (fmol mg^{-1} protein)	n_H
[^{125}I]-ET-1	9	0.33±0.02	12.0±2.9	0.92±0.07
[^{125}I]-S6b	9	0.15±0.01	7.2±1.8	0.95±0.13
[^{125}I]-ET-2	5	1.04±0.23	11.6±1.5	0.93±0.06
[^{125}I]-ET-3	3		–	
[^{125}I]-BQ3020	3		–	

Values are mean±s.e.mean.
– No specific binding detectable at concentrations up to 4 nM.

Table 2 Dissociation constants (K_D), maximal density of receptors (B_{max}) and Hill coefficients (n_H) derived from saturation experiments with the media of human coronary artery

Radioligand	n	K_D (nM)	B_{max} (fmol mg^{-1} protein)	n_H
[^{125}I]-ET-1	3	0.43±0.10	13.1±1.6	0.95±0.03
[^{125}I]-S6b	3	0.27±0.03	17.3±1.8	0.93±0.01
[^{125}I]-ET-2	3	0.71±0.17	14.0±1.8	0.94±0.02
[^{125}I]-ET-3	3	2.13±1.39	20.6±12.9	1.01±0.04
[^{125}I]-PD151242	3	0.23±0.04	10.1±1.2	0.90±0.10
[^{125}I]-BQ3020	3	0.60±0.31	4.5±2.1	0.83±0.06

Values are mean±s.e.mean

Table 3 Dissociation constants (K_D) and maximal densities of receptors (B_{max}) calculated from competition binding assays of [^{125}]-ET-1 (100 pM) vs endothelin receptor ligands in the media of human arteries

Competing ligand	*Coronary artery*		*Aorta*	
	K_D	B_{max} (fmol mg^{-1} protein)	K_D	B_{max} (fmol mg^{-1} protein)
ET-1	ND		0.60±0.20 nM	11.1±1.3
ET-3	ND		8.21±1.62 nM	12.6±1.2
S6b	ND		1.36±0.22 nM	18.6±1.2
ET_A-selective:				
BQ123	*0.85±0.03 nM *7.58±2.27 μM	*15.4±4.1 *1.7±0.5	0.47±0.13 nM	34.9±1.2
FR139317	0.41±0.13 nM	13.5±1.3	0.40±0.10 nM	28.8±1.1
PD151242	3.60±0.74 nM	14.5±1.2	2.09±0.48 nM	35.0±1.2
Non-selective:				
Ro462005	0.19±0.04 μM	12.8±1.5	0.19±0.06 μM	22.8±1.4
Bosentan	2.94±0.95 nM	16.3±1.1	ND	

Values are mean±s.e.mean, n=3 individuals.
*Davenport *et al.*, 1993 (two site fit in coronary artery)
ND, not determined.

However, the more selective ET_A antagonists, FR139317 and PD151242, competed monophasically in coronary artery. ET_B receptors were not detected in the individuals tested with these compounds at concentrations up to 2 μM. The proportion of ET_B receptors indicated by experiments with ET_B-selective agonists was higher than suggested by the highly selective ET_A ligands. This is likely to be due to the lower selectivities of BQ3020 and S6c or a difference in agonist and antagonist binding.

Both of the non-peptide ligands, Ro462005 and bosentan (Clozel *et al.*, 1993; 1994) inhibited [^{125}I]-ET-1 binding monophasically (Table 3), consistent with these antagonists being non-selective. Bosentan demonstrated higher affinity for human coronary artery endothelin receptors than Ro462005.

Autoradiography

Autoradiography was used to examine qualitatively the anatomical distribution of ET_A and ET_B receptors in human aorta and coronary artery. This provided further evidence for the preponderance of ET_A receptors in human vascular smooth muscle (Figure 1). ET_A receptors, visualized by [^{125}I]-PD151242 binding, were identified throughout the smooth muscle of the media of transverse arterial sections. In contrast, little [^{125}I]-BQ3020 binding was apparent in the media, with dense ET_B binding confined to perivascular structures such as adventitial lymphatics and nerves. Such peripheral binding sites were removed, with the adventitia, for the receptor binding assays which were used to characterize only smooth muscle receptors.

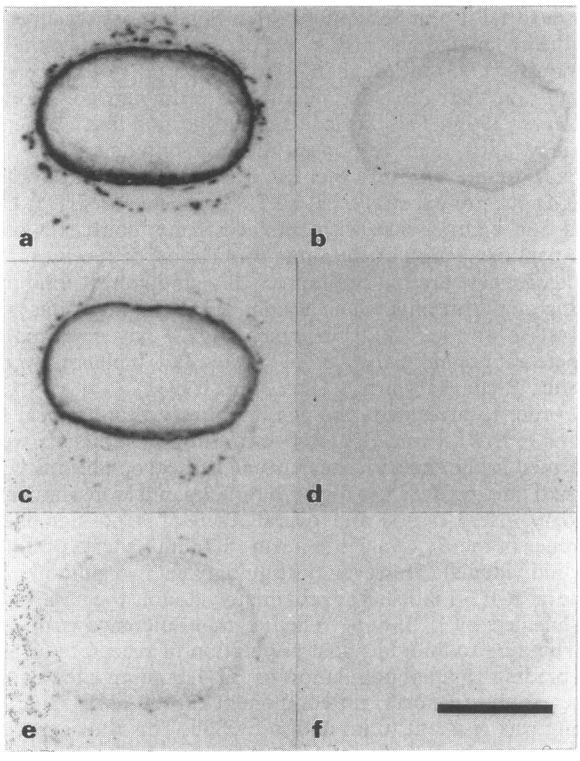

Figure 1 Autoradiograms showing the binding of endothelin receptor subtype selective radioligands in transverse sections of human epicardial coronary artery (scale bar = 2 mm). Total binding of 100 pM [^{125}I]-ET-1, [^{125}I]-PD151242 and [^{125}I]-BQ3020 is shown in panels (a), (c) and (e), respectively. Corresponding non-specific binding images are shown next to each, in panels (b), (d) and (f). ET_A receptors were visualized in the vascular smooth muscle of the media (panel c) with little binding to ET_B receptors in this region (panel e).

Do ET-1 and S6b bind to the same endothelin receptor in human blood vessels?

Maximal binding densities for iodinated ET-1 and S6b were comparable in coronary artery ($n = 3$) (Table 2). The greater amount of smooth muscle obtainable from a larger artery, the aorta, allowed a more detailed direct comparison of [^{125}I]-ET-1 and [^{125}I]-S6b binding with the same nine aortae (Table 1). Overall there was no significant difference in the B_{max} for these radioligands (Student's t test, $P > 0.05$). However, it was noteworthy that in five out of nine aortae, [^{125}I]-ET-1 labelled approximtely double the number of sites labelled by [^{125}I]-S6b.

Increasing concentrations of unlabelled S6b completely inhibited binding of 100 pM [^{125}I]-ET-1 in aortic smooth muscle (Figure 2c). FR139317 was then tested for a difference in ability to compete for binding of [^{125}I]-ET-1, [^{125}I]-ET-2 and [^{125}I]-S6b (Table 4, Figure 2a, 2b). There was no significant difference in the K_D values (Mann-Whitney U test, $P > 0.05$) suggesting that these ligands bind at the same receptors, or that FR139317 is non-selective for further subtypes of the ET_A receptor.

Discussion

Binding studies and autoradiography demonstrate that ET_A receptors are the prevalent endothelin receptor subtype in vascular smooth muscle of human aorta and coronary artery. In aorta, ET_A selective antagonists competed monophasically for the binding of ET-1, ET_B agonists were weak competitors and specific [^{125}I]-ET-3 or [^{125}I]-BQ3020 binding was not detectable. The lack of specific high affinity [^{125}I]-ET-3 or [^{125}I]-BQ3020 binding detectable in aortic smooth muscle is in accord with the absence of a contractile response to these agonists in aorta (Maguire & Davenport, 1995). Unlabelled ET-3 inhibited [^{125}I]-ET-1 binding in aorta with 14 fold lower affinity than unlabelled ET-1, consistent with binding at ET_A receptors. ET-3 has previously been reported to be 30 fold less

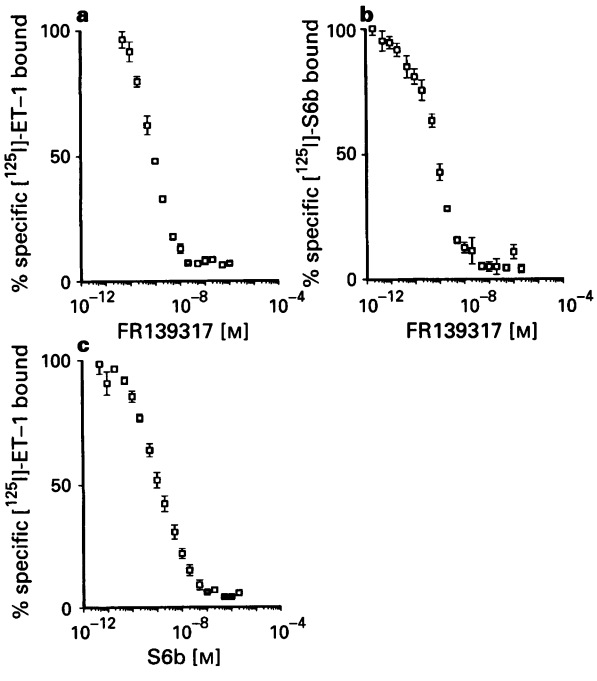

Figure 2 Competition experiments in sections of human aorta. Graphs showing the inhibition of specific binding of: (a) [^{125}I]-ET-1 (100 pM) by FR139317; (b) [^{125}I]-S6b (100 pM) by FR139317 and (c) [^{125}I]-ET-1 (100 pM) by sarafotoxin S6b. Each point represents the mean ± s.e.mean of three individuals. One site fits were preferred on LIGAND analysis of these data.

Table 4 Dissociation constants (K_D) and maximal binding densities (B_{max}) calculated from the inhibition of binding of [^{125}I]-ET-1, [^{125}I]-ET-2 and [^{125}I]-S6b by FR139317 in the media of human arteries

Radioligand	*Coronary artery*		*Aorta*	
	K_D (nM)	B_{max}(fmol mg^{-1} protein)	K_D (nM)	B_{max} (fmol mg^{-1} protein)
[^{125}I]-ET-1	0.41 ± 0.13	13.5 ± 1.3	0.40 ± 0.10	28.8 ± 1.1
[^{125}I]-ET-2	0.98 ± 0.36	23.8 ± 1.2	0.28 ± 0.14	11.1 ± 1.2
[^{125}I]-S6b	0.39 ± 0.13	9.1 ± 1.3	0.72 ± 0.19	5.8 ± 1.2

Values are mean ± s.e.mean, $n = 3$ individuals.

potent than ET-1 at cloned human ET_A receptors (Buchan *et al.*, 1994). In another study, ET_B receptors were detectable in human aorta, albeit in low density accounting for only 11% of receptors (Davenport *et al.*, 1995). This may reflect a difference in the individuals studied: aortae in that study were obtained from patients with ischaemic heart disease.

The B_{max} calculated from [^{125}I]-ET-3 saturation experiments in coronary artery was comparable to that of [^{125}I]-ET-1 and [^{125}I]-ET-2, suggesting interaction with both ET_A and ET_B receptors. The K_D value (2.13 ± 1.39 nM) was lower than that for ET-1 and ET-2 but not as low as would be expected for solely ET_A receptors, implying an intermediate value due to action at both sites. However, detection of two sites over the concentration-range used was not achieved ($n_H = 1.01 \pm 0.04$).

Variability between individuals was seen in the small number of ET_B receptors detected in the media of some coronary arteries. Where present, these represented a small proportion of total endothelin receptors. Previously, competition experiments with BQ123 and 50235 have also indicated a low proportion (10–20%) of ET_B sites in these vessels (Davenport *et al.*, 1993; Maguire *et al.*, 1994a). This variability is paralleled by the similarly inconsistent small constrictor responses to sarafotoxin 6c observed in this tissue *in vitro*. The response to S6c, present in 50% of the coronary arteries tested, reached only 20% of the maximum response to ET-1 (Maguire & Davenport, 1995). Although ET_B receptors in human coronary artery media do not appear to contribute significantly to vasoconstriction, when present they could mediate other uncharacterized effects of endothelin.

Consistent with these findings, ET_A receptors also predominate in other human tissues such as left ventricle and myometrium (Molenaar *et al.*, 1993; Bacon *et al.*, 1995). There is evidence that ET_A is the principal subtype not only in large diameter human arteries but also in smaller resistance vessels. Only ET_A receptors were apparent on the intrarenal arterioles of human kidney viewed by microautoradiography (Karet *et al.*, 1993). Additionally, the ET_A subtype has been demonstrated to mediate ET-1-induced constriction of small diameter, distal segments of human coronary artery (Godfraind, 1993) and human small omental vessels (Riezebos *et al.*, 1994).

Interestingly, K_D values calculated from saturation experiments with [^{125}I]-PD151242 differed from those calculated from competition experiments with the unlabelled ligand (Tables 2 and 3). A similar difference has been observed previously in other studies with human tissue (Davenport *et al.*, 1994a; Peter & Davenport, 1995). A probable explanation is that a structural change accompanying radioiodination enhances the effectiveness of this ligand.

In human coronary artery, the ET_B selective agonists BQ3020 and S6c displayed lower selectivity than previously demonstrated in animals such as the rat (Williams *et al.*, 1991; Ihara *et al.*, 1992). This is likely to be due to species differences; rat and human ET_B receptors have approximately 88% sequence homology (Sakurai *et al.*, 1990; Sakamoto *et al.*, 1991). Also, species variation in the binding characteristics of ET_B agonists has been reported (Nambi & Pullen, 1995; Reynolds *et al.*, 1995). A higher proportion of ET_B receptors estimated from competition experiments with BQ3020 and S6c may be due to lower selectivity or anomalous binding properties of these agonists. Similar results were seen previously with studies on human myometrium (Bacon *et al.*, 1995). The most accurate information would be derived from saturation experiments with BQ3020 which utilize a narrower concentration range (4 pM–4 nM) than competition experiments. These and competition experiments with highly selective ET_A antagonists clearly indicate that ET_B receptors are in the minority in the arteries tested.

Autoradiographic visualization of the anatomical distribution of endothelin receptors in intact aorta and coronary artery confirmed the presence of ET_A receptors throughout the media. Although little ET_B binding was visible on vascular smooth muscle, dense binding was observed in the adventitia on nerves and adventitial lymphatics. In this study, sections used in receptor binding assays were taken only from the smooth muscle layer, where endothelin receptors may mediate vasoconstriction.

Radiolabelled ET-1, ET-2 and sarafotoxin S6b bound to arterial receptors with K_D values in the same range (Tables 1 and 2). Previous studies have indicated that the *in vitro* responses to ET-1 and S6b differ in that S6b is more sensitive to endothelin antagonists (Bax *et al.*, 1993a; Bodelsson & Stjernquist, 1993) although ET-1 and S6b do not differ significantly in their dissociation rates from human aorta (Maguire *et al.*, 1995). This has led to the suggestion that ET-1 and S6b may elicit their responses through different receptors, contrary to the current receptor classification (Sokolovsky, 1994). In the present study, FR139317 competed for ET-1, ET-2 and S6b binding sites with similar affinity, consistent with these peptides acting at the same population of receptors. In both coronary artery and aorta this antagonist inhibited binding of all three ligands in a monophasic manner. This is in contrast to another study in which BQ123 was reported to compete monophasically for ET-1 sites but biphasically for S6b sites in coronary artery (Bax *et al.*, 1993b).

In order to investigate the possible presence of atypical receptors, [^{125}I-ET-1 and [^{125}I]-S6b saturation experiments were performed in the same arteries under identical conditions. The maximal binding densities for both radioligands were similar in coronary artery ($n = 3$) and overall there was no significant difference in the B_{max} values in aorta (Student's t test, $P > 0.05$, $n = 9$ individuals). However, intriguingly in five out of nine aortae [^{125}I]-ET-1 labelled approximately double the number of sites labelled by [^{125}I]-S6b. Whether this difference is due to ET-1 binding to an additional population of receptors or S6b only binding to a subpopulation of ET-1 binding sites awaits further study. In aorta, no component of 100 pM [^{125}I]-ET-1 binding was resistant to inhibition by S6b. This is as expected because the initial phases of [^{125}I]-ET-1 and [^{125}I]-S6b saturation binding curves are superimposable. An S6b-resistant portion of the competition curve might only be evident at a higher concentration of [^{125}I]-ET-1.

Endothelin-induced vasoconstriction is a potential therapeutic target in a number of pathophysiological conditions. The present study demonstrates that endothelin receptors in the media of human arteries are mainly of the ET_A subtype, with a small and variable ET_B population in coronary artery.

The results of this study suggest that ET_A-selective rather than non-selective endothelin receptor antagonists would be most clinically useful. Compounds with this pharmacological profile would block the constrictor ET_A receptors which predominate on human vascular smooth muscle without inhibiting vasodilator ET_B receptors on endothelium. Such agents would also avoid possibly detrimental interactions with ET_B-rich organs such as the kidney (Karet *et al.*, 1993) where either function of receptors is unknown or where they may be important in clearance of endothelin from the circulation.

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