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Characterization of the endothelin receptor selective agonist, BQ3020 and antagonists BQ123, FR139317, BQ788, 50235, Ro462005 and bosentan in the heart

Markus G. Peter & [1]Anthony P. Davenport

Clinical Pharmacology Unit, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ

1 In this study we used ligand binding techniques to determine the affinity and selectivity of endothelin receptor agonists and antagonists in human left ventricle which expresses both ET_A and ET_B receptors, and compared these results with cardiovascular tissues from rat and porcine hearts.

2 The linear tripeptide antagonist, FR139317 competed for [^{125}I]-ET-1 binding to human left ventricle with over 200,000 fold selectivity for the ET_A receptor (K_D $ET_A = 1.20 \pm 0.28$ nM, $K_D ET_B = 287 \pm 93$ μM). The ET_A-selective non-peptide antagonist, 50235, competed with lower affinity and selectivity ($K_D ET_A = 162 \pm 61$ nM, $K_D ET_B = 171 \pm 42$ μM) in this tissue. BQ123 and FR139317 also showed high selectivity (greater than 20,000 fold) and affinity in rat (BQ123: $K_D ET_A = 1.18 \pm 0.16$ nM, $K_D ET_B = 1370 \pm 1150$ μM; FR139317: $K_D ET_A = 2.28 \pm 0.30$ nM, $K_D ET_B = 292 \pm 114$ μM) and pig heart (BQ123: $K_D ET_A = 0.52 \pm 0.05$ nM, $K_D ET_B = 70.4 \pm 4.0$ μM; FR139317: $K_D ET_A = 2.17 \pm 0.51$ nM, $K_D ET_B = 47.1 \pm 5.7$ μM) ($n \geqslant 3$ individuals $\pm$ s.e.mean).

3 Although BQ3020 competed with over 1000 fold selectivity for the ET_B subtype in human heart ($K_D ET_B = 1.38 \pm 0.72$ nM, $K_D ET_A = 2.04 \pm 0.21$ μM) the peptide inhibited only the binding of [^{125}I]-ET-1 at concentrations greater than 100 nM in rat and porcine heart. This is in contrast to the data from the ET_A-selective antagonists which indicated the presence of ET_B sites in these tissues from animal hearts.

4 The peptide antagonist, BQ788, had a low, micromolar affinity ($K_D = 1.98 \pm 0.13$ μM) using human left ventricle and no significant selectivity for the human ET_B-subtype in this tissue.

5 The non-peptide ET antagonists, Ro462005 ($K_D = 50.3 \pm 9.5$ μM) and bosentan (Ro470203; $K_D = 77.9 \pm 7.9$ nM) competed monophasically for [^{125}I]-ET-1 binding sites in human left ventricle.

6 The results show that the ET_A antagonists, BQ123 and FR139317, are highly selective for ET_A receptors in all cardiac tissues tested, whereas BQ788 has a low affinity and no selectivity in this human tissue. Further we showed that there are species differences in the binding of BQ3020 to the ET_B receptors in the hearts derived from human, rat and pig.

Keywords: ET_A and ET_B; left ventricle; BQ3020; BQ123; FR139317; BQ788; 50235; Ro462005; Ro470203; bosentan

Introduction

Endothelin-1 (ET-1) is a potent constrictor of isolated cardiac arteries (Davenport *et al.*, 1989; Godfraind, 1993; Davenport & Maguire, 1994a; Maguire & Davenport, 1995) and a positive inotrope with direct action on the heart muscle *in vitro* (Ishikawa *et al.*, 1988; Qiu *et al.*, 1992) and *in vivo* (Kitayoshi *et al.*, 1989). The *in vitro* positive inotropic effect of endothelin on tissue from failing human heart has an EC_{50}-value in the nM-range (Davenport *et al.*, 1989; Schomisch Moravec *et al.*, 1989; Brodde *et al.*, 1992).

ET-1 and ET-2 are the predominant isoforms detected in heart by radioimmunoassay and high performance liquid chromatography (Plumpton *et al.*, 1993) and immunoreactive ET is localized to endothelial cells of the intramyocardial and epicardial coronary arteries as well as the endocardium (Hemsén *et al.*, 1990; Davenport *et al.*, 1991; Howard *et al.*, 1992; Opgaard *et al.*, 1994).

Two ET receptor sub-types have been cloned from human tissue, ET_A and ET_B. There are species differences between the amino acid sequences encoding the ET receptors present in animals and human subjects. For example, the amino acid sequence for the rat and human ET_A receptors differ by 7% while the rat and human ET_B receptors differ by 12% (Adachi *et al.*, 1991; Ogawa *et al.*, 1991; Arai *et al.*, 1993). Similarly, the deduced amino acid sequence of ET_B receptors from the porcine cerebellum differs by 14% and 13% from that of the rat and human ET_B receptors, respectively (Elshourbagy *et al.*, 1992). The importance of these amino acid differences is unclear making it difficult to compare studies using human and animal tissues (Davenport & Maguire, 1994b).

In view of the species differences, the aim of our study was to use ligand binding techniques to determine the affinity and selectivity of endothelin receptor agonists and antagonists in human left ventricle. We also compared these findings with results obtained in rat and porcine heart. The left ventricular tissue contains a heterogeneous endothelin receptor population ($ET_A : ET_B \approx 60 : 40$; Molenaar *et al.*, 1993; Bax *et al.*, 1993) and is therefore a suitable tissue for determining the affinity and selectivity of new compounds for native human endothelin receptors. A preliminary account of this work has been presented to the British Pharmacological Society (Peter & Davenport, 1994).

Methods

Preparation of cardiac tissues

Human left ventricular free walls were obtained from cardiac allograft recipients at the Papworth Hospital, Cambridge, UK. Patients were all male (28-61 years of age) and drug therapy included calcium antagonists, vasodilators, angiotensin-con-

[1]Author for correspondence at: Clinical Pharmacology Unit, University of Cambridge, Box 110, Level 2, F&G Block, Addenbrooke's Hospital, Cambridge CB2 2QQ.

verting enzyme (ACE) inhibitors, diuretics, digoxin and anticoagulants. The indications for transplantation were ischaemic heart disease, cardiomyopathy or Eisenmengers syndrome. Animal hearts were obtained under laboratory conditions from male Sprague-Dawley rats (200–250 g) and cut into cross-sections which consisted of predominantly left ventricular muscle as well as some right ventricular tissue. Porcine left ventricle were obtained from large white piglets from the same litter. Cardiac tissues were frozen in liquid nitrogen and stored at −70°C. Longitudinal cryostat sections (10 μm) were cut and mounted on microscope slides coated with gelatine and chromic potassium sulphate. Slide mounted tissues were stored at −70°C until further use.

Radioligand binding studies

For competition binding experiments, tissue sections were preincubated in 50 mM HEPES buffer containing 5 mM $MgCl_2$ and 0.3% BSA (pH 7.4) for 15 min at room temperature (22°C). Sections were then transferred to HEPES buffer containing 0.1 nM [^{125}I]-ET-1 in the absence or presence of ET-receptor agonists or antagonists (20 pM to 10 μM) for 120 min at 22°C. Unlabelled ET-1 (1 μM) together with 0.1 nM [^{125}I]-ET-1 was used to define non-specific binding. Sections were then rinsed in Tris buffer (0.05 M Tris-HCl, pH 7.4 at 4°C, 3 × 5 min), scraped from the slide with Whatman GF/C filter paper and counted in a gamma counter (Beckman, Gamma 5500, 77% counting efficiency). Compounds were dissolved in 1% dimethyl-sulphoxide (DMSO) (50235, BQ788, Ro462005, Ro470203), 0.01% ammonia (BQ3020, ET-1), or de-ionised water (BQ123, FR139317); 1% DMSO had no effect on specific binding of [^{125}I]-ET-1 (data not shown).

Protein determination

Protein in sections (10 μm) of left ventricular free wall was determined after solubilization (0.5 M NaOH and 1% sodium dodecyl sulphate for 30 min at 80°C) using the Bio-rad DC 96-well microtiter plate system (Bio-rad Laboratories, Hertfordshire, UK) based on the Lowry method. Microtiter plates were then analysed at 710 nm with a Titertek Multiskan PLUS/MKII (Labsystems, Finland).

Analysis

Binding data were analysed using EBDA (McPherson, 1983) and LIGAND (Munson & Rodbard, 1980) to obtain the dissociation constant (K_D) of the competing ligand and receptor density (B_{max}) values. The K_D of the labelled ligand [^{125}I]-ET-1 was 0.4 nM for human left ventricle (Molenaar *et al.*, 1993), 2.4 nM for pig left ventricle (Peter & Davenport, 1995), and 0.4 nM for rat heart (Bolger *et al.*, 1990).

Data files of several competition curves were run simultaneously with LIGAND to obtain final parameter estimates. The presence of one, two, or three sites was tested using the *F*-ratio test in LIGAND. The model adopted was that which provided the significantly best fit ($P<0.05$).

Drugs

BQ123, cyclo-[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]; BQ3020, [Ala^{11,15}]Ac-ET-1$_{(6-21)}$, FR139317, (N-[(hexahydro-1-azepinyl)carbonyl]L-Leu-(1-Me)D-Trp-3-(2-pyridyl)D-Ala; and BQ788 (N-*cis*-2,6-dimethylpiperidinocarbonyl-L-γMeLeu-D-Trp(CooMe)-D-Nle-ONa) were synthesized by solid phase t-Boc chemistry. Peptide concentration was determined by u.v.-spectrophotometry. Ro462005, 4-*tert*-butyl-N-[6-(2-hydroxy-ethoxy)-5-(3-methoxy-phenoxy)-4-pirimidinyl]-benzenesulphonamide, bosentan (Ro470203), 4-*tert*-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulphonamide, FR139317, and BQ788 were supplied by Dr A.M. Doherty, Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan, U.S.A. 50235, 27-O-Caffeoyl myricerone; was supplied by Shionogi Research Laboratories, Osaka, Japan. [^{125}I]-ET-1 (2000 Ci mmol^{-1}) was obtained from Amersham and unlabelled ET-1 from Novabiochem (Nottingham, U.K.). BSA was from advanced Protein Products Ltd. (Brierley Hill, UK), and all other reagents were purchased from Sigma (Chemical Co., Poole, Dorset, UK).

Results

Competition binding studies with ET_A-selective antagonists

ET_A-selective antagonists, BQ123, FR139317 and 50235, produced biphasic inhibition curves against [^{125}I]-ET-1 in human, rat and porcine heart indicating a heterogeneous receptor population present in each tissue. Two-site fits were preferred to one- or three-site models. BQ123 and FR139317 competed for approximately 60% of the specific [^{125}I]-ET-1 binding sites present in human, rat and porcine heart with dissociation constants (K_DET_A) in the nanomolar range (Table 1 and Figure 1). Selectivity for the ET_A-subtype was in all cases greater than 1000 fold with BQ123 and FR139317. The antagonist,

Table 1 Affinities (K_D) and density of binding sites (B_{max}) for ET receptor selective agonists and antagonists in human, rat and porcine heart tissue

Compound	*Tissue*	n	K_DET_A	K_DET_B	$B_{max}ET_A$ (fmol mg^{-1} protein)	$B_{max}ET_B$ (fmol mg^{-1} protein)	ET_A (%)
FR139317	human†	4	1.20 ± 0.28 nM	> 100 μM	35.7 ± 2.3	21.9 ± 1.9	62
	rat	4	2.28 ± 0.30 nM	> 100 μM	45.2 ± 2.3	27.2 ± 2.2	62
	porcine	3	2.17 ± 0.51 nM	47.1 ± 5.7 μM	657 ± 3	366 ± 3	64
BQ123	human*	3	0.73 ± 0.22 nM	24.3 ± 2.0 μM	56.5 ± 5.1	42.0 ± 1.6	57
	rat	4	1.18 ± 0.16 nM	> 100 μM	46.2 ± 1.7	20.7 ± 1.8	69
	porcine	3	0.52 ± 0.05 nM	70.4 ± 4.0 μM	414 ± 38	186 ± 11	69
BQ3020	human*	3	2.04 ± 0.21 μM	1.38 ± 0.72 nM	(67 ± 3%)	(33 ± 3%)	67
	rat	3	2.03 ± 0.47 μM	> 100 μM	35.8 ± 1.8	25.5 ± 1.4	58
	porcine	3	11.4 ± 1.8 μM	> 100 μM	599 ± 165	149 ± 29	80

Competition binding data for the ET_A receptor-selective antagonists, BQ123 and FR139317, and the ET_B receptor-selective agonist, BQ3020. Slide mounted heart tissue sections were incubated with 100 pM [^{125}I]-ET-1 in the presence or absence of (10^{-5}–10^{-12} M) ligand for 2 h. Data were derived from EBDA and LIGAND analysis. Data marked (*) were taken from Molenaar *et al.* (1993) and data marked (†) were taken from Peter & Davenport (1995) for comparison. In some cases, owing to the high selectivity of the ligands for the ET_A receptor, values for the low affinity ET_B site could not be accurately estimated and are shown as greater than 100 μM.

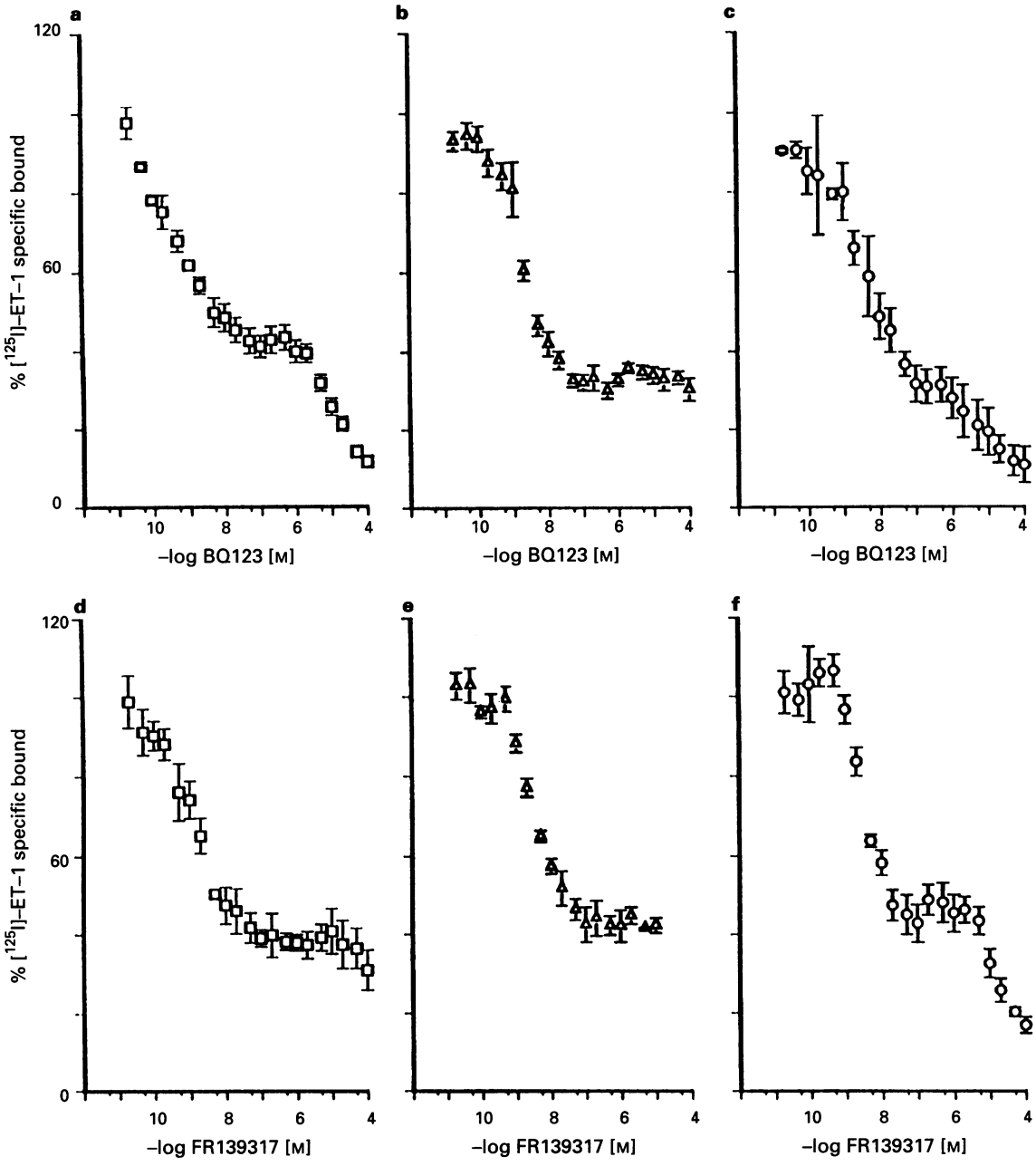

Figure 1 Competitive binding of 100 pM [^{125}I]-ET-1 to slide-mounted tissue sections of human and porcine left ventricle and rat heart by unlabelled BQ123 and FR139317. Inhibition curves are biphasic suggesting the presence of two [^{125}I]-ET-1 binding sites. BQ123 (a, b, c) and FR139317 (d, e, f) show similar competition curves for all species with $K_D ET_A = 0.5-2$ nM and $K_D ET_B > 20$ μM. Human left ventricle (□), rat heart (△), porcine left ventricle (○).

50235, also indicated the presence of two binding sites in human left ventricle (ET_A : ET_B 55 : 45) with a 1000 fold selectivity for the ET over the ET_B receptor subtype (Table 2 and Figure 2b). However, the compound had a 100–200 fold lower affinity for ET_A receptors than BQ123 and FR139317 (Tables 1,2 and Figures 1a,d, 2b).

Competition binding studies with ET_B-selective ligands

The ET_B-selective agonist, BQ3020, produced a biphasic inhibition curve when competing against [^{125}I]-ET-1 (1,500 fold selectivity for the ET_B over the ET_A subtype, Table 1 and Figure 2d) in the human heart. However, in rat and porcine heart, BQ3020 competed for [^{125}I]-ET-1 binding sites only at high concentrations (0.1–100 μM) (Table 1, Figures 2e, f). In human left ventricle BQ788 competed only at concentrations above 0.1 μM with a K_D in the micromolar range. Analysis of the monophasic competition curve by LIGAND indicated that a one-site fit was preferred to models containing multiple sites (Table 2 and Figure 2c).

Competition binding studies with non-selective ligands

The competition binding curve for the non-peptide ET antagonist, Ro462005 against [^{125}I]-ET-1 in human left ventricle was monophasic (Figure 2a). Analysis of the binding data confirmed that a one-site model with a K_D of 50 μM was preferred to a two-site fit (Table 2). Bosentan had a 600 fold higher affinity for ET-receptors than Ro462005 (K_D = 78 nM, Table 2, Figure 2a).

Table 2 Affinities (K_D) and density of binding sites (B_{max}) for ET receptor selective and non-selective compounds in human left ventricular tissue

Compound	n	K_D ET_A (nM)	K_D ET_B (μM)	B_{max} ET_A (fmol mg^{-1} protein)	B_{max} ET_B (fmol mg^{-1} protein)
50235	3	162±61	171±42	28.1±4.2	22.9±3.3
		$K_DET_{A/B}$		$B_{max}ET_{A/B}$ (fmol mg^{-1} protein)	
Ro462005	4	50.3±9.5 μM		74.9±13.2	
Bosentan	3	77.9±7.9 nM		56.1±7.6	
BQ788	6	1.98±0.13 μM		51.4±7.4	
ET-1	3	0.74±0.24 nM		91.7±3.2	

Equilibrium dissociation constants (K_DET) and maximal densities of receptors (B_{max} ET) for subtype selective and non-selective ET-receptor antagonists in human left ventricular free wall. 50235 is a non-peptide ET_A receptor antagonist with a hundred fold lower affinity for ET_A receptors than the peptide antagonists FR139317 and BQ123, and indicates a proportion of ET_A receptors of approximately 55%.

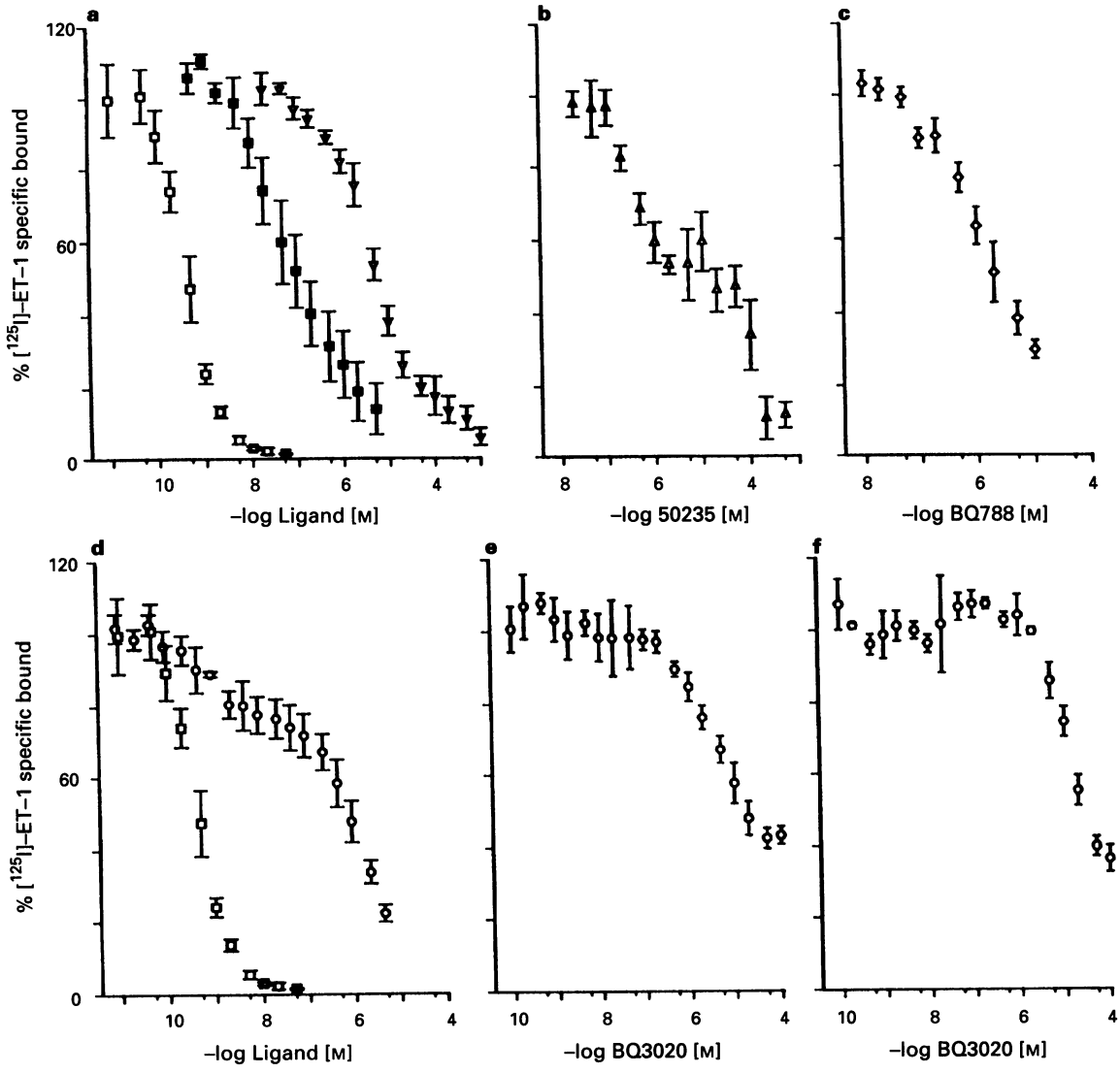

Figure 2 Competitive binding of [^{125}I]-ET-1 to tissue sections of human left ventricle (a, b, c, d), rat heart (e), and porcine left ventricle (f) by unlabelled ET-1 (□), the non-peptide antagonists Ro462005 (▽), bosentan (■) and 50235 (△), the putative ET_B-selective antagonist BQ788 (◇), and the ET_B-selective agonist BQ3020 (○). The inhibition curves for Ro462005, bosentan, ET-1 (a) and BQ788 (c) on human left ventricle are monophasic indicating non-selective binding. In contrast, the inhibition curves for 50235 (b) and BQ3020 (d) on human left ventricle are biphasic suggesting the precence of two [^{125}I]-ET-1 binding sites. BQ3020 competes with specific [^{125}I]-ET-1 binding sites from rat and porcine heart (e, f) in the micromolar range. Results are expressed as percentage of specific binding (n=3–6, mean±s.e.mean).

Discussion

We have characterized the binding of selective and non-selective ligands to endothelin receptors in human, rat and porcine heart. These binding studies demonstrated that BQ123 and FR139317 are highly selective for the ET_A stubtype in all three species with similar sub-nanomolar affinities as the endogenous agonist ligand ET-1. These results are in agreement with affinities reported for FR139317 in bovine ET_A transfected CHO cells ($K_i = 1$ nM; Aramori *et al.*, 1992). Further, our calculated affinities for the cyclic pentapeptide BQ123 are also similar to those reported for porcine cultured aortic smooth muscle cells expressing ET_A receptors ($IC_{50} = 7.3$ nM, Ihara *et al.*, 1992a), porcine cardiac ventricular membranes (IC_{50}: 1.8 nM; Kikuchi *et al.*, 1994), as well as for cloned human ET_A receptors expressed in transfected COS-7 cells ($K_i = 2.4$ nM; Webb *et al.*, 1992) or baby hamster kidney cells (Hechler *et al.*, 1993). Previous studies in various tissues and cultures are in agreement that BQ123 has more than 1000 fold selectivity as we found in human heart. However, a range of affinities for the ET_A receptor subtype have been reported, with for example an IC_{50} of approximately 90 nM calculated by Bax *et al.* (1993) for native human ET_A and by Sakamoto *et al.* (1993) for COS-7 cells expressing human ET_A receptors ($IC_{50} \approx 25$ nM). The K_D values reported for BQ123 in binding assays are lower than the affinities (K_B values) derived from functional assays. Other ET_A selective ligands may also show a further discrepancy but the reasons for this remain unclear (Maguire *et al.*, 1995a, b). In the human heart we have shown that FR139317 is slightly more selective for ET_A than for ET_B receptors compared with the cyclic pentapeptide BQ123 (Molenaar *et al.*, 1993; Peter & Davenport, 1995). These results also indicate that the tripeptide structure of FR139317 is sufficient to inhibit binding of the 21 amino acid peptide to the ET_A-subtype. Although the affinity of 50235 is about 100 fold less than BQ123, the results demonstrate that the carbon-nitrogen backbone is not essential for inhibition of binding to ET_A receptors. The dissociation constant of 50235 for the ET_A receptor subtype in the human left ventricle is in the high nanomolar region, similar to the affinity reported for rat cardiac membranes ($K_i = 78$ nM; Fujimoto *et al.*, 1992) and rat aortic smooth muscle A7r5 cells which express ET_A receptors ($K_i = 51$ nM; Mihara & Fujimoto, 1993).

In human left ventricle FR139317, BQ123 and 50235 revealed a similar ratio of ET_A to ET_B receptors (approximately 60:40%) (Molenaar *et al.*, 1993; Bax *et al.*, 1993; Peter & Davenport 1995; and present study). A similar proportion of ET_A-receptors (60–67%) was found in porcine and rat cardiac tissues with BQ123 and FR139317. The density of receptors labelled with [^{125}I]-ET-1 was similar in human subjects and rats: about 70–75 fmol mg^{-1} protein. The density in pig heart was 10 fold higher and this is likely to reflect the neonatal source of the tissue.

Human left ventricle contains a heterogeneous population of cells. We have previously shown that the ET_A subtype accounts for more than 90% of endothelin receptors in isolated myocytes (Molenaar *et al.*, 1993). In addition, we have found that ET_A receptors predominate in the medial layer (which contains mainly smooth muscle cells) of intramyocardial vessels (Davenport *et al.*, 1993; 1995). ET_B receptors are likely to be localized to other cell types such as endothelial cells (Tsukahara *et al.*, 1994) and probably neuronal tissue and fibroblasts (Katwa *et al.*, 1993). Although ET_A receptors are the predominant subtype in the vasculature (Davenport *et al.*, 1993; 1995), in other human tissues ET_B receptors are more abundant than ET_A receptors (70% in human kidney, Karet *et al.*, 1993).

The ET_B-ligand, BQ3020, is a linear truncated peptide analogue of ET-1 (Saeki *et al.*, 1991) and has been shown to bind selectively to human ET_B receptors in kidney (Karet *et al.*, 1993), heart (Molenaar *et al.*, 1993) and in the media from aorta and pulmonary and coronary arteries (Davenport *et al.*, 1993). In contrast, the competition binding data for porcine left ventricle and rat heart show that BQ3020 inhibits [^{125}I]-ET-1 binding in these tissues only at concentrations greater than 0.1 μM (Table 1). Thus, under the binding conditions used here, BQ3020 did not compete with high affinity for [^{125}I]-ET-1 in these animal hearts, although the presence of ET_B sites was implied by using ET_A antagonists. The inhibition constants of BQ3020 in rat and porcine tissues appear to be fairly similar to those calculated for the ET_A-subtype in human tissue (approximately 2 μM). In addition, BQ3020 competed against the ET_A-selective radioligand [^{125}I]-PD151242 (Davenport *et al.*, 1994) in rat heart with a dissociation constant (approximately 2.5 μM; data not shown) similar to that found for human left ventricle ($K_D = 1.5 \pm 0.3$ μM; Peter & Davenport, 1995) and rat brain ($K_D = 2.7 \pm 0.9$ μM; Nandasoma & Davenport, 1994). We were able to confirm the previous observation by Molenaar *et al.* (1993) in the human heart showing similar binding affinity constants and receptor subtype ratios for BQ3020 when competing for [^{125}I]-ET-1 binding sites ($K_D ET_B = 2.35 \pm 1.18$ nM, $K_D ET_A = 2.88 \pm 0.35$ μM; ratio ET_B/ET_A: 30/70). These experiments using animal tissue were carried out in parallel with human left ventricle suggesting that batch-variation of BQ3020 was not the source of the observed binding discrepancies. However, experiments with the ET_B-selective radioligand, [^{125}I]-IRL1620, demonstrated that binding was less reversible from rat tissues than from the human tissues studied, also indicating species differences in the binding characteristics of the ET_B subtype (Nambi *et al.*, 1994).

The discrepancies in the binding affinities for BQ3020 in human heart and in rat and porcine hearts may be explained by differences in the primary sequences of ET_B receptors in these species. The amino acid sequence for the rat and human ET_A receptors differs only by 7% while the rat and human ET_B receptors differs by 12% (Adachi *et al.*, 1991; Ogawa *et al.*, 1991; Arai *et al.*, 1993) and the deduced amino acid sequence of ET_B receptors from the porcine cerebellum differs by 13% from that of the human ET_B receptor (Elshourbagy *et al.*, 1992). However, this does not explain why BQ3020 competes for [^{125}I]-ET-1 binding sites in porcine cerebellar membranes (ET_B receptors) with high affinity (IC_{50}: 0.2 nM; Ihara *et al.*, 1992b), with high and low affinity sites in rat cerebellum ($K_D ET_B = 41.5$ nM, $K_D ET_A = 10.3$ μM; Davenport *et al.*, 1992) but only low affinity sites in rat and porcine ventricle. These results suggest that ET_B receptors may be modified according to the tissue in which they are expressed. Similar conclusions were drawn from functional experiments, where it was shown that haemodynamic responses to ET-1 and BQ3020 in conscious rats are differentially affected not only depending on the dose of agonist, but also according to the tissue region (Gardiner *et al.*, 1994). In addition, studies in rat showed that ET receptors in the coronary blood vessels are of a different type (neither ET_A nor ET_B) from those in other vascular beds (Gulati *et al.*, 1995): Pretreatment with BQ123 completely blocked a decrease in blood flow to the heart induced by Sarafotoxin 6b, but did not affect the decrease in blood flow to other organs induced by Sarafotoxin 6b. Although recombinant rat and human ET_B receptors expressed in CHO-K1 cells had similar affinities for BQ3020 when competing against [^{125}I]-ET-3 (0.2 nM), they had different affinities when competing against several peptide and non-peptide antagonists (Reynolds *et al.*, 1995). Thus despite their high degree of homology, pharmacological differences have been observed for rat, porcine and human ET_B receptors, between the different species as well as between different tissues within the same animal model. This might explain the different binding profile of BQ3020 when competing against [^{125}I]-ET-1 in rat and porcine ventricle as compared to those from human subjects in the present study.

BQ788 has been described previously as a potent and selective ET_B receptor antagonist by inhibiting [^{125}I]-ET-1 binding to ET_B receptors on transformed human Girardi heart cells (IC_{50}: 1.2 nM; Ishikawa *et al.*, 1994) and to rabbit arterial pulmonary membranes (Fukuroda *et al.*, 1994). However, in the current study, BQ788 competes only in a non-selective way

at concentrations greater than 0.1 μM when competing with [^{125}I]-ET-1 for the mixed $ET_{A/B}$ receptor population present in human left ventricle. We also showed previously that another putative ET_B-selective compound, IRL1038, has only a low affinity in the micromolar range and poor selectivity for the human ET_B receptor in the human left ventricle ($K_D ET_B = 6$ μM, $K_D ET_A = 38$ μM; ratio ET_B/ET_A: 38/62; Peter & Davenport, 1994). Initially, this compound was reported to have a nanomolar affinity for the ET_B receptor in membrane preparations of animal tissues (Urade *et al.*, 1992) but more recently, questions have arisen about the reproducibility of these data (Urade *et al.*, 1994) and IRL1038 may not be as potent as originally thought.

The non-peptide antagonist, Ro462005, completely inhibited the specific binding of [^{125}I]-ET-1 to human left ventricle, but was effective only in the μM-range with an IC_{50} of 6.3 ± 4.3 μM, which is slightly higher than the concentrations reported to be necessary with human cultured vascular smooth muscle cells (0.22 μM) and rat aortic endothelial cells (1 μM) (Clozel *et al.*, 1993). Bosentan demonstrated greater than 600 fold higher affinity for specific [^{125}I]-ET-1 binding sites in the human heart than Ro462005. This was similar to that reported for bosentan in human smooth muscle (ET_A) and human placenta (ET_B): K_i of 4.7 nM and 95 nM, respectively (Clozel *et al.*, 1994). Although oral administration of bosentan decreased mean arterial blood pressure in a conscious rat coronary heart failure model (Teerlink *et al.*, 1994), and reduced elevated blood pressure, vascular hypertrophy and remodelling in DOCA-salt (deoxycorticosterone acetate-salt) hypertensive rats (Li *et al.*, 1994), bosentan did not affect myocyte or coronary endothelial injury in a rat model of ischaemia and reperfusion (Richard *et al.*, 1994).

We have previously characterized the mRNA encoding the ET receptors present in human left ventricle using molecular biology techniques (Molenaar *et al.*, 1993). In all individuals examined, RT-PCR (reverse transcriptase-polymerase chain reaction) assays demonstrated a single band corresponding to the expected position for mRNA encoding the ET_A and a single band corresponding to the ET_B subtype. On sequencing the PCR products, they were the expected sequence corresponding to the two known subtypes. Using these oligonucleotide primers, we have not detected the presence of additional bands which might suggest further subtypes. Also, using the ligands tested in this study (Peter & Davenport, 1995), we did not detect any additional binding sites which might have suggested the presence of further endothelin receptor subtypes in the human heart.

In conclusion, we have determined the affinity and selectivity of endothelin selective ligands in human, rat and porcine cardiac tissue. We have demonstrated a similar high affinity and selectivity of ET_A receptors in these tissues for BQ123 and FR139317. Studies with 50235, Ro462005 and bosentan indicate that the carbon-nitrogen backbone is not essential for non-peptide antagonist binding to endothelin receptors in human left ventricle. ET_B-selective ligands showed only unsatisfactory binding parameters: BQ788 bound only with low affinity and poor selectivity to the ET_B subtype in human tissues, and further, the binding profile for BQ3020 differed markedly for human tissue and rat and porcine heart tissue. Our binding studies suggest, together with *in vitro* functional data from other groups, that cardiovascular ET_B receptors from rat and pig may differ from those in other vascular beds in other species, for example in man. On the other hand, we could not detect any species differences in the binding profile of ET_A antagonists. Although the reason for the differences in ET_B-binding is unclear, it shows that extrapolating onto human subjects from data derived from animal studies should be done with caution. We also demonstrated that sections of human left ventricle containing a heterogeneous population of native human receptors are a useful tool for characterization of ligand-receptor interactions in the endothelin system.

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