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Characterization of the binding of endothelin ET_B selective ligands in human and rat heart

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1 We determined competition binding characteristics of endothelin ET_B receptor selective ligands in human left ventricle and compared these values to those obtained with rat left ventricle. Sarafotoxin S6c, ET-3, BQ788 and IRL2500 competed against [^{125}I]-BQ3020 (ET_B selective radioligand; Molenaar *et al.*, 1992) with high affinity and against [^{125}I]-PD151242 (ET_A selective radioligand) with low affinity in human left ventricle, confirming the ET_B selectivity of these compounds.

2 ET-3 competed with moderate selectivity for ET_B over ET_A receptors in human left ventricle and with slightly higher selectivity in rat left ventricle (460 and 1,400 fold, respectively). There was a small difference in the affinity of ET_A receptors for ET-3 (K_D ET_A in human left ventricle = 0.07 ± 0.02 μM; K_D ET_A in rat left ventricle = 0.27 ± 0.08 μM; $P = 0.05$) but no difference in the affinity of ET_B receptors for this ligand (K_D ET_B in human left ventricle = 0.15 ± 0.06 nM; K_D ET_B in rat left ventricle = 0.19 ± 0.03 nM).

3 The selectivity of sarafotoxin S6c for ET_B over ET_A receptors in human left ventricle was 5,900 fold compared with 59,400 fold in rat left ventricle. The affinity of ET_A receptors for sarafotoxin S6c was higher in human than in rat left ventricle (K_D $ET_A = 2.00 \pm 0.20$ μM and 3.50 ± 0.26 μM, respectively; $P = 0.03$), while the affinity of ET_B receptors for this ligand was higher in rat left ventricle (K_D $ET_B = 0.06 \pm 0.02$ nM) than in human left ventricle (K_D $ET_B = 0.34 \pm 0.13$ nM) ($P = 0.02$). The affinity of ET_B receptors for sarafotoxin S6c in rat left ventricle determined in the absence or presence of GTP was the same indicating that differing affinity states of ET_B receptors in human and rat left ventricle do not account for the variation observed between species.

4 There was no difference in the affinity of ET_A receptors for BQ788 (K_D $ET_A = 1.01 \pm 0.20$ μM and K_D $ET_A = 1.39 \pm 0.35$ μM) or for the novel ET_B selective antagonist, IRL2500 (K_D $ET_A = 30.0 \pm 20.8$ μM and K_D $ET_A = 55.6 \pm 9.93$ μM) in human and rat left ventricle, respectively. ET_B receptors had a significantly higher affinity for BQ788 (K_D $ET_B = 9.8 \pm 1.3$ nM and K_D $ET_B = 31.0 \pm 5.4$ nM; $P = 0.02$) and IRL2500 (K_D $ET_B = 78.2 \pm 9.7$ nM and K_D $ET_B = 300.0 \pm 75.1$ nM; $P = 0.03$) in human and rat left ventricle, respectively. The synthetically synthesized ET_B selective antagonist RES-701-1 (0.1-3 μM) failed to inhibit [^{125}I]-ET-1 binding in either tissue.

5 In conclusion, we have compared equilibrium dissociation constants for a number of ET_B selective compounds in human and rat heart. The affinity of ET_B receptors for sarafotoxin S6c, BQ788 and IRL2500 differed in human and rat left ventricle. No difference in affinity was detected for ET-3 binding at ET_B receptors. Sarafotoxin S6c binding was unaffected by GTP indicating that the different receptor affinities in human and rat heart cannot be explained by differing ET_B receptor affinity states. This study highlights the need to consider differences in binding characteristics that may arise from the use of tissues obtained from different species.

Keywords: Endothelin; ET_A receptors; ET_B receptors; sarafotoxin S6c; ET-3; BQ788; IRL2500; RES-701-1

Introduction

Endothelin (ET) receptors have been characterized in a number of human and animal tissues and cell types. ET_A receptors predominate in the smooth muscle layer of the human vasculature (greater than 85%; Davenport *et al.*, 1995) and mainly mediate ET-1 induced vasoconstriction (Maguire & Davenport, 1995). Activation of endothelial ET_B receptors is thought to produce vasorelaxation (Cockcroft *et al.*, 1991), while ET_B receptor activation in the smooth muscle produces a small and variable vasoconstrictor response (Maguire & Davenport, 1995). In contrast, in some animal species including the rat there is a significant ET_B constrictor component (Davenport & Maguire, 1994).

ET_B-selective ligands have been important in determining the contribution of this receptor subtype to the modulation of vascular contractility and relaxation. Human *in vitro* and *in vivo* studies are sometimes based on affinity and selectivity ratios determined in animal tissue despite evidence to suggest that the binding characteristics of some endothelin selective ligands are species-dependent. Rat and human ET_B receptors differ by 12% in amino acid sequence and although this represents a high degree of homology, important pharmacological differences have been noted. The dissociation of [^{125}I]-BQ3020 and [^{125}I]-IRL1620 from ET_B receptors was less marked in rat membranes than in canine membranes or CHO cells transfected with a human ET_B receptor clone (Nambi *et al.*, 1994; Nambi & Pullen, 1995). The binding characteristics of a number of ligands including the ET_B-selective antagonists RES-701-1 and BQ788 were also found to be species-dependent (Tanaka *et al.*, 1995; Reynolds *et al.*, 1995).

Human vascular preparations contain ET_A and ET_B receptors but the proportion of ET_B receptors is small. A homogeneous tissue preparation that expresses a high proportion of both ET_A and ET_B receptors can be used to determine an accurate estimate of receptor affinity and ligand selectivity. This avoids possible discrepancies that can arise from the use of different tissues or from the use of cell types that may undergo different post-translational modifications to the native receptors. The aim of this study was to determine the binding characteristics of ET_B selective ligands in human left

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ventricle, a tissue that contains ET_A and ET_B receptors in a ratio of about 7:3, and to compare these values with that determined in the corresponding animal tissue.

Methods

Collection of tissues

Left ventricle was obtained from patients (8 male and female, 23–64 years) with idiopathic dilated cardiomyopathy (IDCM) undergoing cardiac transplantation at the Papworth Hospital (Cambridgeshire, U.K.). Drug therapy included angiotensin converting enzyme inhibitors, corticosteroids, β_2-adrenoceptor agonists, cardiac glycosides, diuretics, anticoagulants and calcium antagonists. Left ventricle was also obtained from American Cancer Institute (ACI) rats (17 male and female, 170–290 g) killed by carbon dioxide. Tissues were frozen immediately in liquid nitrogen and stored at −70°C.

Saturation binding studies

Sections of human left ventricle (10 μm) were cut on a cryostat (Bright, Huntingdon, U.K.) at −22°C and then preincubated in humidified chambers with HEPES buffer (HEPES 50 mM, $MgCl_2$ 5 mM, BSA (fraction V) 0.3%; pH 7.4) for 15 min at 23°C. Excess HEPES buffer was removed from the slides before labelling sections with increasing concentrations of [^{125}I]-ET-1 (non-selective), [^{125}I]-PD151242 (ET_A selective; Davenport *et al.*, 1994) or [^{125}I]-BQ3020 (ET_B selective; Molenaar *et al.*, 1992) (2 h, 23°C). Non-specific binding was determined at each concentration by 1 μM of the corresponding unlabelled ligand. Sections were washed in Tris buffer (Tris 0.05 M, pH 7.4 at 4°C, 3 × 5 min) wiped from the slides with Whatman GF/C filters and counted in a gamma counter (Beckman 5500, 79% efficiency).

Competition binding studies

Sections of human and rat left ventricle were preincubated with HEPES buffer and then labelled with either [^{125}I]-ET-1, [^{125}I]-PD151242 or [^{125}I]-BQ3020 (0.1 nM, 2 h, 23°C) in the presence of increasing concentrations of ET_B selective compounds. Non-specific binding was determined in the presence of 1 μM of the corresponding unlabelled ligand. The affinity of ET_A receptors for the ligands was determined from two site fits of the [^{125}I]-ET-1 binding data. In a second series of experiments the ability of ET_B selective compounds to compete for [^{125}I]-ET-1 binding was tested in the presence of FR139317 (0.1 μM), which is highly selective for ET_A receptors in human and rat ventricle (Sogabe *et al.*, 1992). This concentration of FR139317 was calculated to inhibit more than 95% of ET_A receptors and less than 1% of ET_B receptors in order to examine binding to ET_B receptors. Sections were washed, wiped from the slides and counted.

Protein determination

The amount of protein in sections (10 μm) of left ventricle was determined after solubilization in 0.5 M NaOH and 1% sodium dodecyl sulphate (30 min, 80°C) by the Bio-rad DC 96-well microtiter plate system (Bio-rad Laboratories, Hertfordshire, U.K.) and based on the Lowry method. Microtiter plates were then analysed at 710 nm with a Titertek Multiskan PLUS/MK11 (Labsystems Oy, Finland).

Analysis of data

Initial estimates of receptor affinity (K_D) and the maximum density of binding sites (B_{max}) were obtained with EBDA software (McPherson, 1983) with final estimates determined by use of LIGAND (Munson & Rodbard, 1980). The equilibrium dissociation constants determined for each radioligand by saturation analysis were used to determine binding parameters for the endothelin selective competing ligands. The partial F test was used to determine the presence of one or two site fits and was supported by the determination of pseudo Hill coefficients. Results are expressed as the mean ± s.e.mean and significance, defined by a P value less than 0.05, was determined by the Mann-Whitney U test. The selectivity of the ligands for one subtype over the other was determined with ET_B receptor affinity values obtained from competition against [^{125}I]-ET-1 in the presence of FR139317.

Drugs

BQ3020 ([Ala^{11,15}]Ac-ET-1$_{(6\text{-}21)}$) and FR139317 ((N-[(hexahydro-1-azepinyl)carbonyl)L-Leu-(1-Me)D-Trp-3-(2-pyridyl)D-Ala) were synthesized by solid phase t-Boc chemistry. Peptide concentration was determined by uv-spectrophotometry. IRL2500 (N-(3,5-dimethylbenzoyl)-N-methyl-(D)-(4-phenyl phenyl)alanyl-(L)-tryptophan) was supplied by Dr M. Shiono, International Research Laboratories (Takarazuka, Japan). FR139317, PD151242 ((N-[(hexahydro-1-azepinyl)carbonyl]L-Leu(1-Me)D-Trp-D-Tyr) and BQ788 (N-cis-2,6-dimethyl piperidinocarbonyl L-γ-MeLeu-D-Trp(COOCH$_3$)-D-Nle) were supplied by Dr A.M. Doherty, Parke-Davis Pharmaceutical Research Division (Ann Arbor, Michigan, U.S.A.). RES-701-1 (Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp-OH) was from Calbiochem Novabiochem (Beeston, Nottingham, U.K.). [^{125}I]-ET-1, [^{125}I]-PD151242 and [^{125}I]-BQ3020 (2000 Ci mmol^{-1}) were from Amersham International plc. (Amersham, U.K.). ET-1 and ET-3 were from Novabiochem (Nottingham, U.K.), sarafotoxin S6c was from Peninsula Laboratories, Inc. (Belmont, California, U.S.A.). All other reagents were from Sigma Chemical Co. (Poole, Dorset, U.K.), BDH (Poole, Dorset, U.K.) or Boehringer Mannheim GmbH (Mannheim, Germany).

Results

Saturation binding in human left ventricle revealed [^{125}I]-ET-1 binding to ET_A and ET_B receptors with a single equilibrium dissociation constant (Table 1). A one site fit was preferred to a two site fit for the binding of [^{125}I]-PD151242 to ET_A receptors and for the binding of [^{125}I]-BQ3020 to ET_B receptors (Table 1). The mean maximum % of specific binding for [^{125}I]-ET-1, [^{125}I]-PD151242 and [^{125}I]-BQ3020 was 72% at 20 pM, 95% at 1 nM and 76% at 20 pM, respectively. Non-specific binding was determined with 1 μM of the corresponding unlabelled ligands. A one site fit was preferred to a two site fit for [^{125}I]-ET-1 binding in rat left ventricle ($K_D = 0.39 \pm 0.01$ nM, $n_H = 0.96$ and $B_{max} = 129.7 \pm 2.1$ fmol mg^{-1} protein, $n = 3$).

We next tested the ability of unlabelled compounds to compete for the binding of iodinated ET_A and ET_B selective radiolabelled ligands. Sarafotoxin S6c, ET-3, BQ788 and IRL2500 competed against [^{125}I]-BQ3020 at ET_B receptors in human left ventricle with sub-nanomolar to nanomolar affinity

Table 1 Equilibrium dissociation constants (K_D), maximal density of binding sites (B_{max}) and Hill coefficients (n_H) determined for [^{125}I]-ET-1, [^{125}I]-BQ3020 and [^{125}I]-PD151242 in human left ventricular tissue sections

Radioligand	K_D (nM)	B_{max} (fmol mg^{-1} protein)	n_H	n
[^{125}I]-ET-1	0.10 ± 0.01	40.8 ± 6.0	0.99	3
[^{125}I]-BQ3020	0.10 ± 0.02	10.9 ± 1.9	1.09	3
[^{125}I]-PD151242	0.25 ± 0.31	46.8 ± 2.0	1.07	3

Data shown are means ± s.e.mean; n = number of patients.

Table 2 Equilibrium dissociation constants (K_D ET_A and K_D ET_B) and maximal density of receptors (B_{max} ET_A and B_{max} ET_B) for endothelin selective compounds competing against [^{125}I]-PD151242 (0.1 nM) and [^{125}I]-BQ3020 (0.1 nM) binding in human left ventricular tissue sections

	[^{125}I]-PD151242 binding			*[^{125}I]-BQ3020 binding*		
Competitor	K_D ET_A (μM)	B_{max} ET_A (fmol mg^{-1} protein)	n	K_D ET_B (nM)	B_{max} ET_B (fmol mg^{-1} protein)	n
S6c	2.05±0.13	34.8±3.9	5	0.10±0.03	7.2±1.5	5
ET-3	0.06±0.01	39.7±1.6	5	0.04±0.01	9.7±1.6	8
BQ788	0.54±0.10	27.1±2.4	5	12.0±3.4	4.2±0.7	5
IRL2500	10.0±0.7	42.7±1.9	4	64.8±16.3	8.4±1.1	7

Data shown are means±s.e.mean; n=number of patients.

Table 3 Equilibrium dissociation constants (K_D ET_A) and maximal densities of receptors (B_{max} ET_A) for ET_B selective compounds competing against [^{125}I]-ET-1 (0.1 nM) binding sites in human and rat left ventricular tissue sections

	Human	*Human*		*Rat*	*Rat*	
Competitor	K_D ET_A (μM)	B_{max} ET_A (fmol mg^{-1} protein)	n	K_D ET_A (μM)	B_{max} ET_A (fmol mg^{-1} protein)	n
S6c	2.00±0.20	29.1±1.4	6	3.50±0.26*	57.8*	4
ET-3	0.07±0.02	27.5±2.2	4	0.27±0.08	35.5±4.7	7
BQ788	1.01±0.20	14.2±2.4	5	1.39±0.35	37.7±3.7	4
IRL2500	30.0±20.8	25.3±5.5	5	55.6±9.9	41.0±4.6	3
RES-701-1	ND	ND	4	ND	ND	3

ND, competition for RES-701-1 (0.1–3.0 μM) against [^{125}I]-ET-1 was not detected; n=number of patients or animals. * Sarafotoxin S6c competed in a monophasic manner against [^{125}I]-ET-1 in rat left ventricle and so the K_D of this ligand at ET_A receptors may be lower than that shown. * B_{max} was derived by subtracting B_{max} ET_B (11.4 fmol mg^{-1} protein; Table 4) from the total B_{max} (69.2±4.5 fmol mg^{-1} protein).

and against [^{125}I]-PD151242 at ET_A receptors with submicromolar to micromolar affinity (Table 2), confirming the ET_B selectivity of these compounds in this tissue.

[^{125}I]-ET-1, which labels both ET_A and ET_B receptors and is the iodinated form of the endogenous ligand, was used in subsequent studies. All compounds competed against [^{125}I]-ET-1 in a biphasic manner in both tissues except sarafotoxin S6c in rat left ventricle which was best described by a one site fit. Sections were labelled with [^{125}I]-ET-1 in the presence of the ET_A selective antagonist, FR139317 (0.1 μM) to determine accurate estimates of ET_B receptor affinity. A two site fit was preferred to a one site fit with the partial F test and was confirmed by pseudo Hill coefficients that were less than unity (values between 0.53 and 0.77 in human left ventricle and between 0.31 and 0.63 in rat left ventricle for sarafotoxin S6c, ET-3 and BQ788). Residual binding insensitive to inhibition by FR139317 represented breakthrough of the ET_A receptor blockade and was a small proportion of ET_A receptors in human left ventricle (between 6.0 and 16.1%) and rat left ventricle (between 21.5 and 27.6%); data not shown. IRL2500 competed in a monophasic manner in both human and rat left ventricle (n_H=1.01 and 1.03, respectively).

The affinity of ET_A receptors for ET-3 was higher in human than in rat left ventricle, although this difference was small (P=0.05; Table 3). There was no difference in the affinity of ET_B receptors for ET-3 in the two tissues as expected. ET-3 competed for the binding of [^{125}I]-ET-1 with moderate selectivity for ET_B over ET_A receptors in human left ventricle (460 fold) and with slightly higher selectivity in rat left ventricle (1400 fold).

Sarafotoxin S6c competed against [^{125}I]-ET-1 binding at ET_A receptors with slightly higher affinity in human than in rat left ventricle (P=0.03; Figure 1; Table 3), while the affinity of ET_B receptors for sarafotoxin S6c was higher in rat than in human left ventricle (P=0.02; Figure 2; Table 4). The affinity of ET_B receptors for sarafotoxin S6c and density of ET_B receptors in rat left ventricle (K_D ET_B=0.06±0.02 nM; B_{max}=12.3±1.1 fmol mg^{-1} protein) was unchanged by the inclusion of guanosine 5′-triphosphate (GTP) in the preincubation buffer and labelling solution (K_D ET_B=0.07±0.03 nM; B_{max}=16.8±1.2 fmol mg^{-1} protein). The selectivity of sarafotoxin S6c for ET_B over ET_A receptors was 5,900 fold in human left ventricle and 59,400 fold in rat left ventricle (Figure 2).

There was no difference in the affinity of ET_A receptors for either BQ788 or for the novel ET_B selective antagonist, IRL2500 in the two tissues (Table 3). The affinity of ET_B receptors for BQ788 and IRL2500 was higher in human than in rat left ventricle (P=0.02 and 0.03, respectively; Table 4). RES-701-1, an ET_B selective antagonist, failed to inhibit [^{125}I]-ET-1 binding in human and rat left ventricle at concentrations up to 3 μM. RES-701-1 (10 μM) inhibited specific [^{125}I]-ET-1 binding in human left ventricle by less than 20% but failed to inhibit binding in rat left ventricle.

B_{max} values tended to be slightly lower when determined by competition against [^{125}I]-BQ3020 binding (Table 2) than against [^{125}I]-ET-1 binding (Table 4), although this did not reach significance. The affinity of ET_B receptors for sarafotoxin S6c, BQ788 and IRL2500 was similar in competition against [^{125}I]-BQ3020 and [^{125}I]-ET-1. ET-3 competed with a slightly but significantly higher affinity (3.7 fold, P=0.01) at ET_B receptors against [^{125}I]-BQ3020 than against [^{125}I]-ET-1. The proportion of ET_A to ET_B receptors in human left ventricle determined for each competing ligand was 72.4/27.6% (sarafotoxin S6c), 70.0/30.0% (ET-3), 60.7/39.3% (BQ788) and 65.9/34.1% (IRL2500) while in rat left ventricle the ratio was 83.5/16.5% (sarafotoxin S6c), 73.8/26.2% (ET-3), 76.6/23.4% (BQ788) and 74.0/26.0% (IRL2500).

Discussion

The present study examines the binding characteristics of ET_B selective compounds in human and rat left ventricle. We have used left ventricle obtained from patients undergoing cardiac transplantation for idiopathic dilated cardiomyopathy where the proportion of ET_B to ET_A receptors is in agreement with previously obtained values in human left ventricle (Molenaar *et al.*, 1993; Bax *et al.*, 1993).

[^{125}I]-ET-1 (non-selective), [^{125}I]-PD156707 (ET_A selective) and [^{125}I]-BQ3020 (ET_B selective) labelled receptors in a

monophasic manner in saturation binding experiments. Whilst the level of [^{125}I]-ET-1 binding would be expected to be higher than [^{125}I]-PD156707 binding we found no significant difference. Although all tissues were obtained from patients with dilated cardiomyopathy this discrepancy may be explained by the use of unpaired tissue samples.

The affinity of ET-3 for ET_B receptors was similar in human and rat left ventricle and similar to that obtained in human uterus (K_D = 0.24 nM; Maggi *et al.*, 1994). Sarafotoxin S6c inhibited [^{125}I]-ET-1 binding in human left ventricle with a selectivity ratio for ET_B over ET_A receptors (5,900 fold) that was similar to that found for human prostatic tissue (1,700 fold; Kobayshi *et al.*, 1994) and CHO cells transfected with human ET_A or ET_B receptors (4,000 fold; Buchan *et al.*, 1994). This was less than the selectivity determined in rat left ventricle (59,400 fold) and less than that found in rat uterus, a tissue containing predominantly the ET_A receptor subtype and rat hippocampus, a tissue containing predominantly ET_B re-

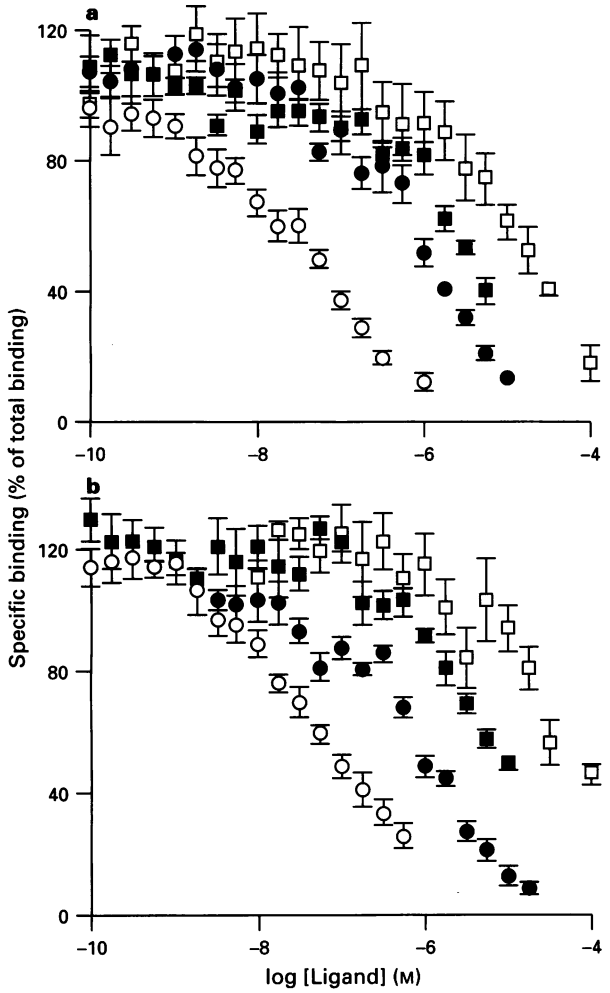

Figure 1 Binding curves for the competition of ET_B selective ligands against [^{125}I]-ET-1 in human (a) and rat (b) left ventricular tissue sections. Curves were biphasic with the exception of sarafotoxin S6c in rat left ventricle which was monophasic. The affinity of ET_A receptors for the ligands was determined by analysis of the low affinity component of the curves (see Table 3). Sarafotoxin S6c (■), IRL2500 (□), BQ788 (●), ET-3 (○).

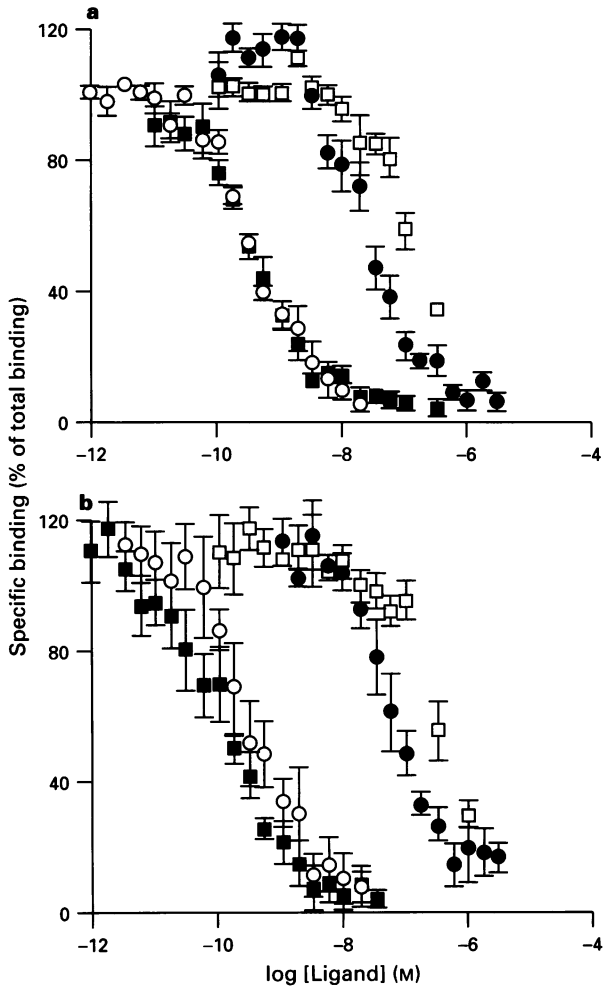

Figure 2 Binding curves for the competition of ET_B selective ligands against [^{125}I]-ET-1 in the presence of the ET_A selective antagonist, FR139317, in human (a) and rat (b) left ventricular tissue sections. A small amount of residual, low affinity binding was observed that represented breakthrough of the ET_A antagonist blockade. ET_B receptor affinity was determined by analysis of the high affinity component of the curves (see Table 4). Sarafotoxin S6c (■), IRL2500 (□), BQ788 (●), ET-3 (○).

Table 4 Equilibrium dissociation constants (K_D ET_B) and maximal densities of receptors (B_{max} ET_B) for ET_B selective compounds competing against [^{125}I]-ET-1 (0.1 nM) binding sites in the presence of the ET_A selective antagonist FR139317 (0.1 μM) in human and rat left ventricular tissue sections

Competitor	*Human* K_D ET_B (nM)	*Human* B_{max} ET_B (fmol mg^{-1} protein)	n	*Rat* K_D ET_B (nM)	*Rat* B_{max} ET_B (fmol mg^{-1} protein)	n
S6c	0.34±0.13	11.1±2.3	5	0.06±0.02	11.4±0.6	4
ET-3	0.15±0.06	11.8±3.2	5	0.19±0.03	12.6±1.9	3
BQ788	9.81±1.32	9.2±3.4	5	31.0±5.41	11.5±0.9	4
IRL2500	78.2±9.7	13.1±2.9	4	300.0±75.1	14.4±0.8	4
RES-701-1	ND	ND	4	ND	ND	4

ND, competition for RES-701-1 (0.1–3.0 μM) against [^{125}I]-ET-1 was not detected; n = number of patients or animals.

ceptors (>174,000 fold; Williams *et al.*, 1991). The differences in selectivity ratios in human and rat left ventricle can be attributed to differences in ET_A and ET_B receptor affinity for sarafotoxin S6c. The affinity of ET_B receptors for sarafotoxin S6c in human left ventricle (Table 4) and canine femoral vein (K_i=0.025 nM; Miller & Michener, 1995) also differ markedly. This difference is unlikely to be tissue rather than species dependent as binding of sarafotoxin S6c to blood vessels in human meningiomas (IC_{50}=0.23 nM; Yamaga *et al.*, 1995) was similar to our results in human left ventricle.

There remained the possibility that the differences in ET_B receptor affinity for sarafotoxin S6c in human and rat left ventricle could be attributed to differing affinity states of the receptors in the two tissues. Rat left ventricular tissue sections were preincubated with GTP to test this hypothesis. There was no change in receptor affinity or density induced by the presence of GTP indicating that differing affinity states of the ET_B receptors do not account for the differences observed between species in this study. The failure of GTP to convert [^{125}I]-ET-1 binding sites from a high to a low affinity state in the present study is consistent with the inability of 5'-guanyly-imidophosphate (GppNHp), a non-hydrolysable analogue of GTP, to convert [^{125}I]-ET-1 binding sites in rat atrial membranes (Sokolovsky, 1995).

The affinity of BQ788 and IRL2500 for ET_B receptors was slightly but significantly higher in human than in rat left ventricle. BQ788 competed at ET_B receptors in human left ventricle with 4 and 8 fold lower affinity than in cultured human umbilical vein endothelial cells and cultured human Girardi heart cells, respectively (Ishikawa *et al.*, 1994; Ozaki *et al.*, 1995). In contrast, ET_A receptors in human left ventricle (Table 1) and human SK-N-MC cells (Ishikawa *et al.*, 1994) had similar micromolar affinity indicating that the differences in binding in native human endothelin receptors and to endothelin receptors in cultured cells may pertain only to the ET_B receptor subtype.

RES-701-1 is an ET_B selective antagonist first isolated from *Streptomyces* sp. RE-701 (Morishita *et al.*, 1994; Tanaka *et al.*, 1994) but it is the synthetic compound that is commercially available and used for ET_B receptor characterization. Synthetic RES-701-1 was found to have very low affinity for ET_B receptors, consistent with a recent study which examined the ability of isolated and synthetic RES-701-1 to compete for [^{125}I]-ET-1 binding in CHO cells stably transfected with the human ET_B receptor (He *et al.*, 1995). Here the affinity of ET_B receptors for isolated RES-701-1 was in the low nanomolar range whereas the affinity for the synthesized compound was in the low micromolar range. A recent study suggests that isolated and synthetic RES-701-1 differ in their tertiary structure (Yano *et al.*, 1995).

[^{125}I]-BQ3020 has previously been found to differentiate affinity states of ET_B receptors (Jarvis *et al.*, 1994), and it has recently been proposed that [^{125}I]-BQ3020 may be a specific ligand for picomolar affinity sites (Sokolovsky, 1995). High and low affinity binding sites were described in a saturation study with [^{125}I]-BQ3020 (highest concentrations greater than 0.1 nM) in rat cerebellar membranes (Jarvis *et al.*, 1994). It is possible that the slightly lower density of ET_B receptors detected by competition against [^{125}I]-BQ3020 compared to [^{125}I]-ET-1, although not significant, is a reflection of preferential binding to one of two populations of binding sites. However, it is likely that picomolar affinity binding sites represent a minor proportion of the ET_B receptors in human left ventricle as these were not detected by [^{125}I]-BQ3020 saturation binding (0.01–1.1 nM; n_H=1.09).

Although there exists a high degree of sequence homology for human and rat ET_B receptors, important pharmacological differences have been noted. The potential for pharmacological heterogeneity in ET_B receptors differing by over 50 amino acids, such as rat and human ET_B receptors, is substantial. For example, substitution of only one amino acid, lysine-181 to aspartic acid, in the rat ET_B receptor caused a marked decrease in affinity for ET-1, ET-2, ET-3 and S6c (Mauzy *et al.*, 1992; Zhu *et al.*, 1992). However, in this instance lysine-181 is conserved in rat and human ET_B receptors and is unlikely to be involved in the species differences. The greatest variation in amino acid sequence is in the amino-terminal extracellular domain. Although a third of the rat and human amino acid sequence differs here, it is unlikely to be important in differences in agonist affinities observed between the species since cleavage of the amino-terminal domain from partially purified human placental ET_B receptor had no effect on ET-1, ET-2 or ET-3 binding (Akiyama *et al.*, 1992), indicating that this region is not essential for ligand binding. Exchange of the amino-terminal extracellular tails of the ET_A and ET_B receptors also failed to alter binding characteristics of ET-1, ET-3 or BQ123 (Sakamoto *et al.*, 1993). The fourth, fifth and sixth transmembrane domains and adjacent loop regions have been shown to be important to ET_B selective agonist binding (Sakamoto *et al.*, 1993; Becker *et al.*, 1994), and so this region may be central to the heterogeneity observed with sarafotoxin S6c.

In this study ET_A receptors had low affinity and ET_B receptors had moderate to high affinity for sarafotoxin S6c, BQ788, IRL2500 and ET-3 in human and rat left ventricle, while synthetic RES-701-1 (0.1–3 μM) failed to bind to either subtype. ET_B receptors were found to have different affinities for sarafotoxin S6c, BQ788 and IRL2500 but not for ET-3. These differences indicate that the binding characteristics of ET_B selective ligands determined in animal tissue may not be predictive of values that are obtained in human tissue. This study highlights the need for consideration of species differences in the use of ET_B selective compounds for the characterization of ET_B receptors in human tissues.

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