Biochemical Pharmacology, Vol. 58, pp. 369–374, 1999.

ELSEVIER

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00081-7

Endothelin Receptors in Adult Human and Swine Isolated Ventricular Cardiomyocytes

Pietro Amedeo Modesti,‖ Simone Vanni,* Rita Paniccia,* Avio Perna,* Massimo Maccherini,† Gianfranco Lisi,† Guido Sani†§ and Gian Gastone Neri Serneri**

*Clinica Medica e Cardiologia, University of Florence; †Institute of Thoracic and Cardiovascular Surgery, University of Siena; and §Department of Cardiosurgery, University of Cagliari, Italy

ABSTRACT. The present study aimed to investigate endothelin-1 (ET-1) receptors in human and swine cardiomyocytes with binding studies using ET_A and ET_B selective receptor antagonists (BMS-182874 and BQ-788, respectively). Cell distribution of mRNA expression for ET_A and ET_B subtypes was investigated by *in situ* hybridization using specific cDNA probes. The ^{125}I-ET-1 binding, which reached equilibrium in about 120 min (K_{obs} = 0.051 ± 0.003 min^{-1}), was only partially displaceable by the addition of a large excess of ET-1 (about 15% with a half-life of 20 min). In equilibrium binding studies, ^{125}I-ET-1 had a K_d of 0.43 ± 0.08 nM and a maximum binding (B_{max}) of 42.8 ± 6.6 fmol/mg protein. ET_A and ET_B receptors are represented in human and swine cardiomyocytes with an 85:15 ratio as indicated by the biphasic pattern of competition of both BMS-182874 and BQ-788. *In situ* hybridization studies confirmed that myocytes mainly expressed mRNA for ET_A, whereas expression of mRNA for the ET_B subtype was documented in non-myocyte cells. These results showed that ET-1 binds with high affinity and poor reversibility to specific receptors, in both human and swine isolated ventricular cardiomyocytes, without significant species differences. BIOCHEM PHARMACOL **58**;2: 369–374, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. endothelin; cardiomyocytes; ET_A receptor; ET_B receptor; BMS-182874; BQ-788

Endothelin-1 binds to two high-affinity specific membrane receptors, ET_A** and ET_B [1, 2]. Endothelin ET_A receptor exhibited a rank order of binding affinities of ET-1 > ET-2 ≫ ET-3, whereas endothelin ET_B receptor showed comparable affinities for all three ET peptides. Both receptor subtypes are similarly represented in left human heart with an endothelin ET_A/ET_B ratio of 60% to 40% [3]. In animal models, ventricular cardiomyocytes mainly express the endothelin ET_A receptor subtype [4, 5] whereas other cardiac cell lines, such as fibroblasts and endothelial cells, express both ET_A and ET_B [4, 6, 7], but no studies have been performed in normal human left ventricular myocytes. Although the primary sequence of ET receptors is largely conserved, species differences have been reported both in the amino acid sequence, 7% and 12% between rat and human for ET_A and ET_B respectively, and in binding affinity for selective antagonists [8–12]. These findings indicate that extrapolation of data derived from studies performed in small animals onto human subjects should be undertaken with caution. The pig has certain unique properties which render it attractive for heart research, including a marked resemblance to humans in terms of anatomy of coronary circulation and response to growth factors [13, 14]. Furthermore, studies are in progress to use large animals for cardiac xenotransplantation [15]. The present study was therefore performed to define the kinetic properties, density, affinity, and subtype distribution of ET-1 receptors in human and swine left ventricular cardiomyocytes using selective ET_A (BMS-182874) [16] and ET_B antagonists (BQ-788) [17], and non-selective ET_A/ET_B receptor antagonist (PD-145065). In addition, the *in vivo* distribution of endothelin receptor subtypes was investigated by *in situ* hybridization studies on left ventricular tissue using specific cDNA probes.

MATERIALS AND METHODS

Tissue Procurement

Human myocardial tissue was obtained from five healthy donors (aged 54 ± 7 years) whose hearts were not used for transplantation because of body size or age. The study was conducted according to the Helsinki declaration of human rights [18]. Four male farm pigs were used in the present study. Preoperative weights ranged from 30 to 40 kg

‖ Corresponding author: Pietro Amedeo Modesti, M.D., Ph.D., Clinica Medica I, University of Florence, Viale Morgagni 85, 50134 Florence, Italy. Tel. ++39 055 432758; FAX ++39 055 4378638; E-mail: pa.modesti@dfc.unifi.it

** *Abbreviations:* B_{max}, maximum binding; ET-1, endothelin-1; ET_A, endothelin type A receptor; ET_B, endothelin type B receptor; and MEM modified Eagle medium.

Received 9 July 1998; accepted 12 January 1999.

(average wt 35 ± 3 kg). The surgical procedures were conducted according to the guidelines of the European law on animal use. Animals were premedicated with intramuscular ketamine (15 mg/kg) and diazepam (5 mg/kg). Anesthesia was induced with sodium pentobarbital (20 mg/kg ev) and maintained with a mixture of 1% to 1.5% fluothane and oxygen.

Cell Isolation

After explantation, the heart was placed in ice-cold cardioplegic solution (NaCl 82 mM, KCl 20 mM, $CaSO_4$ 0.5 mM, $MgSO_4$ 7.5 mM, $NaHCO_3$ 23 mM, glucose 25 mM, mannitol 55 mM) and immediately transferred to the laboratory. The left coronary artery was cannulated and perfused with calcium-free buffer (MEM) Eagle Joklik (Sigma Chemicals) with 21 mM HEPES, 4.4 mM $NaHCO_3$, 1.5 mM KH_2CO_4, 1.7 mM $MgCl_2$, 11.7 mM glucose, 2 mM L-glutamine, 21 U/mL insulin (pH 7.2) (HEPES-MEM buffer) gassed with 95% O_2 and 5% CO_2 at 32° for 10 min (blood washout). Collagenase perfusion was carried out at 32° with HEPES-MEM buffer gassed with 95% O_2 and 5% CO_2 and Worthington type II collagenase 100 U/mL (20 mL/min) for 30 min. After digestion, the portion of left ventricle was minced and shaken in resuspension buffer (HEPES-MEM buffer supplemented with BSA 0.5%, 0.3 mM $CaCl_2$, 10 mM taurine) and Worthington type II collagenase 100 U/mL for 30 min at 37°. Intact cells were enriched by centrifuging the resuspended pellet through Percoll (Pharmacia Fine Chemicals) at 35 *g* for 10 min. Receptor binding studies were performed the same day on freshly suspended cells (10^5 cells/mL) at room temperature. Immunocytochemical staining performed using mouse monoclonal antibody against human myosin (Sigma Chemicals, M8421) demonstrated that the cell preparation was almost pure (more than 99%).

Binding Studies

KINETIC ANALYSIS. The kinetics of association of ^{125}I-ET-1 (100 pM, final concentration 2000 Ci/mmol, Amersham) to isolated cardiomyocytes (10^5 cells/mL) at 22° in a final volume of 0.2 mL was evaluated at selected times (30 sec to 240 min). Non-specific binding was measured as the binding obtained by adding unlabeled ET-1 (1 μM, final concentration). The incubation mixture was then rapidly filtered under reduced pressure through Whatman GF/C glass microfiber filters presoaked with polyethylene glycol 6.6%. The kinetic of dissociation was evaluated by adding unlabeled ET-1 (1 μM, final concentration) to the reaction mixture after 120 min of incubation. The residual binding was measured at selected times (30 sec to 120 min). Kinetic constants (K_{obs}, K_{-1}, and K_1) were calculated according to Weiland and Molinoff [19].

EQUILIBRIUM STUDIES AND IDENTIFICATION OF ENDOTHELIN RECEPTOR SUBTYPES. In equilibrium binding studies, isolated cardiomyocytes (10^5 cells/mL) were incubated with ^{125}I-ET-1 (100 pM) and increasing concentrations of either unlabeled ET-1 (0–1 μM), PD-145065 (0–10 μM), BMS-182874 (0–1 mM), or BQ-788 (0–1 mM) for 120 min at 22° in a final volume of 0.2 mL. Incubation was stopped and samples were processed as described above. The inhibitory constants for ET-1, PD-145065, BMS-182874, and BQ-788 upon the ^{125}I-ET-1 binding to isolated cardiomyocytes were preliminarily calculated according to Cheng and Prusoff [20]. Competition binding data were then analyzed by iterative curve fitting to a one- or two-site binding model using a non-linear-fitting computer program (LIGAND) to obtain the final estimation of K_d for ET-1, K_i for PD-145080, BMS-182874 and BQ-788, and the receptor density (B_{max}) values [21].

In Situ *Hybridization Studies*

cDNA probes for ET_A and ET_B receptor subtypes were prepared from the phage clones of human endothelin receptors (ET_A, American Type Culture Collection, ATCC 105194 and ET_B, ATCC 1250426). *In situ* hybridization studies were performed as previously described [22]. Positive controls were obtained for each sample using a cDNA probe for the constitutively expressed D-glyceraldehyde-3-phosphate dehydrogenase (ATCC 57090) to ensure that mRNA in myocardial biopsies was intact. To ensure the specificity of the *in situ* hybridization signals, we performed the following negative control studies: sections were tested with hybridization mixture (1) without the probe and (2) after incubation with RNAase A (1 Kunitz unit per liter) for 1 hr at 37° before hybridization. *In situ* hybridization staining was performed at the same time for all specimens and at least twice on serial sections in each specimen.

Statistical Analysis

Each experiment was performed in triplicate. If not otherwise indicated, all data given in the text are means ± SD.

RESULTS

Kinetic Studies

Kinetic studies revealed that the association of ^{125}I-ET-1 with human cardiomyocytes was specific but not readily reversible (Fig. 1). ^{125}I-ET-1 binding reached steady state by 120 min and remained almost stable for the following 4 hr. The observed association constant (K_{obs}) was 0.051 ± 0.003 min^{-1}. The addition of a 10,000-fold excess of unlabeled ET-1 only partially displaced ^{125}I-ET-1 specific binding (15%) with a $t_{1/2}$ of 20 min. The dissociation rate constant (K_{-1}) was 0.034 ± 0.007 min^{-1} with a resulting K_1 of 0.159 ± 0.049 nM^{-1} min^{-1}. The kinetically derived dissociation constant was 0.22 ± 0.033 nM (Fig. 1). Using myocyte membranes instead of intact cells, we obtained the

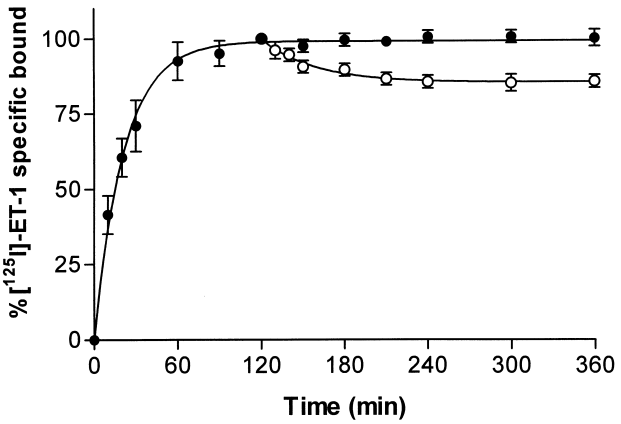

FIG. 1. Time-course of the association (●) and dissociation (○) phase of ^{125}I-ET-1 specific binding to human isolated cardiomyocytes. Each point represents the mean ± SD.

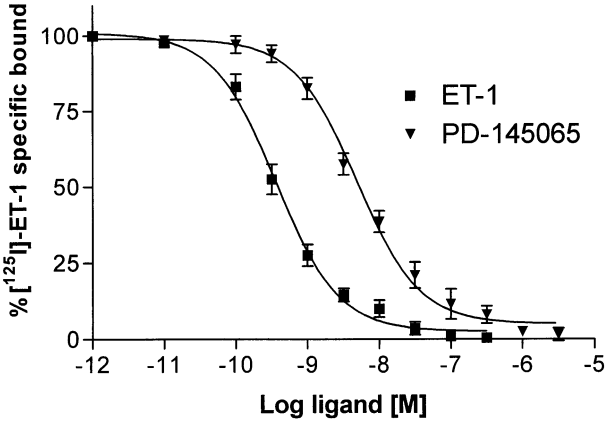

FIG. 2. Competition experiments using isolated cardiomyocytes. Effects of ET-1 and PD-145065 on specific ^{125}I-ET-1 binding. Binding is expressed as the percentage of specific binding in the absence of competitors. Each point represents the mean ± SD.

same data, suggesting that cell uptake is not the basis for the poor reversibility of ^{125}I-ET-1 binding. Experiments performed in swine cardiomyocytes showed a similar time pattern with a kinetically derived dissociation constant of 0.15 ± 0.012 nM.

Competition Experiments

Specific ^{125}I-ET-1 binding to intact human and swine left ventricular cardiomyocytes accounted for 80–85% of the total binding and was a linear function of the number of cells added to the binding reaction between 10^5 and 5×10^5 cells/mL. Scatchard analysis of binding data yielded a straight line in both human and swine cells, with an affinity in the low nanomolar range. In human cells, the K_d was 0.43 ± 0.08 nM with a B_{max} of 42.8 ± 6.6 fmol/mg protein, corresponding to 43,724 ± 6,530 binding sites/cell. ET receptors of swine cardiomyocytes had a similar affinity and B_{max} (Table 1). Both unlabeled ET-1 and PD-145065 displaced ^{125}I-ET-1 binding in a monophasic manner, with Hill coefficients close to the unit (Fig. 2). Conversely, the best fitting of competition binding data for BMS-182874 and BQ-788 was obtained with a two-component binding model, indicating the presence of a small percentage of ET_B receptors (about 15% for both antagonists) (Fig. 3). Both BMS-182874 and BQ-788 were highly selective, because the two mean inhibitory constants (K_i) were 73 ± 23 nM (ET_A) and >100 μM (ET_B) for BMS-182874 (Table 1) and 318 ± 81 nM and 0.63 ± 0.03 nM (ET_A and ET_B, respectively) for BQ-788. Competition in pig cardiomyocytes yielded similar inhibitory constants (Table 1).

In Situ *Hybridization Studies*

In human hearts, negative and positive controls showed that the hybridization was specific for mRNA and that the mRNA in the left ventricular tissue specimens was intact (Fig. 4, A and B). In human hearts, mRNA for ET_A receptors was expressed in both the myocytes and non-

TABLE 1. Competition studies of [^{125}I]-ET-1 binding to human and swine isolated cardiomyocytes

	ET-1	PD-145065	BMS-182874	BQ-788
Human cardiomyocytes				
B_{max} (fmol mg^{-1})	42.8 ± 6.6	–	–	–
K_d (nM)	0.43 ± 0.08	6.6 ± 1.7	–	–
n_H	0.97	0.93	0.57	0.49
ET_A (%)	–	–	85 ± 2	86 ± 3
K_i	–	–	73 ± 23 nM	318 ± 81 nM
ET_B (%)	–	–	15 ± 2	14 ± 2
K_i	–	–	>100 μM	0.63 ± 0.03 nM
Swine cardiomyocytes				
B_{max} (fmol mg^{-1})	38.5 ± 9.2	–	–	–
K_d (nM)	0.24 ± 0.09	9.7 ± 2.6	–	–
n_H	0.94	0.95	0.51	0.48
ET_A (%)	–	–	87 ± 3	87 ± 4
K_i	–	–	85 ± 28 nM	327 ± 22 nM
ET_B (%)	–	–	13 ± 2	14 ± 2
K_i	–	–	>100 μM	0.58 ± 0.4 nM

n_H, Miu coefficient.

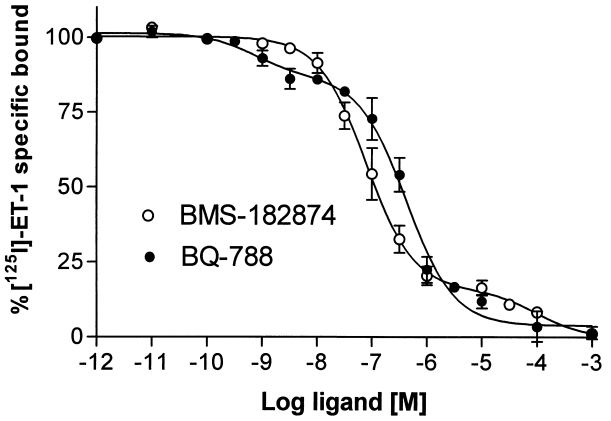

FIG. 3. Competition experiments using isolated cardiomyocytes. Effects of BMS-182874 and BQ-788 on specific ^{125}I-ET-1 binding. Binding is expressed as the percentage of specific binding in the absence of competitors. Each point represents the mean ± SD.

myocyte cells (Fig. 4C). In contrast, mRNA for ET_B receptors was almost exclusively expressed in fibroblasts and endothelial cells (Fig. 4D). Similar results were obtained in swine hearts.

DISCUSSION

The present results indicate that ET_A is the prevalent (85%), but not the only, receptor subtype expressed by normal human cardiomyocytes, since selective ET_A (BMS-182874) and selective ET_B antagonists (BQ-788) displaced the ^{125}I-ET-1 binding with a non-linear pattern showing the presence of a small percentage (15%) of ET_B receptors. Cardiac ET-1 activities are mediated by two endothelin receptor subtypes, ET_A and ET_B, both represented in human left ventricular myocardium [23] with an average proportion of 60:40 (ET_A:ET_B) [3]. The prevalent expression of ET_B by interstitial cells (endothelial cells and fibroblasts) and the shared distribution of ET_A subtype between myocyte and non-myocyte cardiac cells in human and porcine hearts is well documented in *in situ* hybridization studies. The discrepancy between the almost absent mRNA signal for the ET_B subtype in cardiomyocytes at *in situ* hybridization and the 15% of ET_B receptors detected at binding studies in the same cell type might be attributed to two factors. Firstly, the messenger and the protein of ET_B receptors might have a different half-life and turn-over and

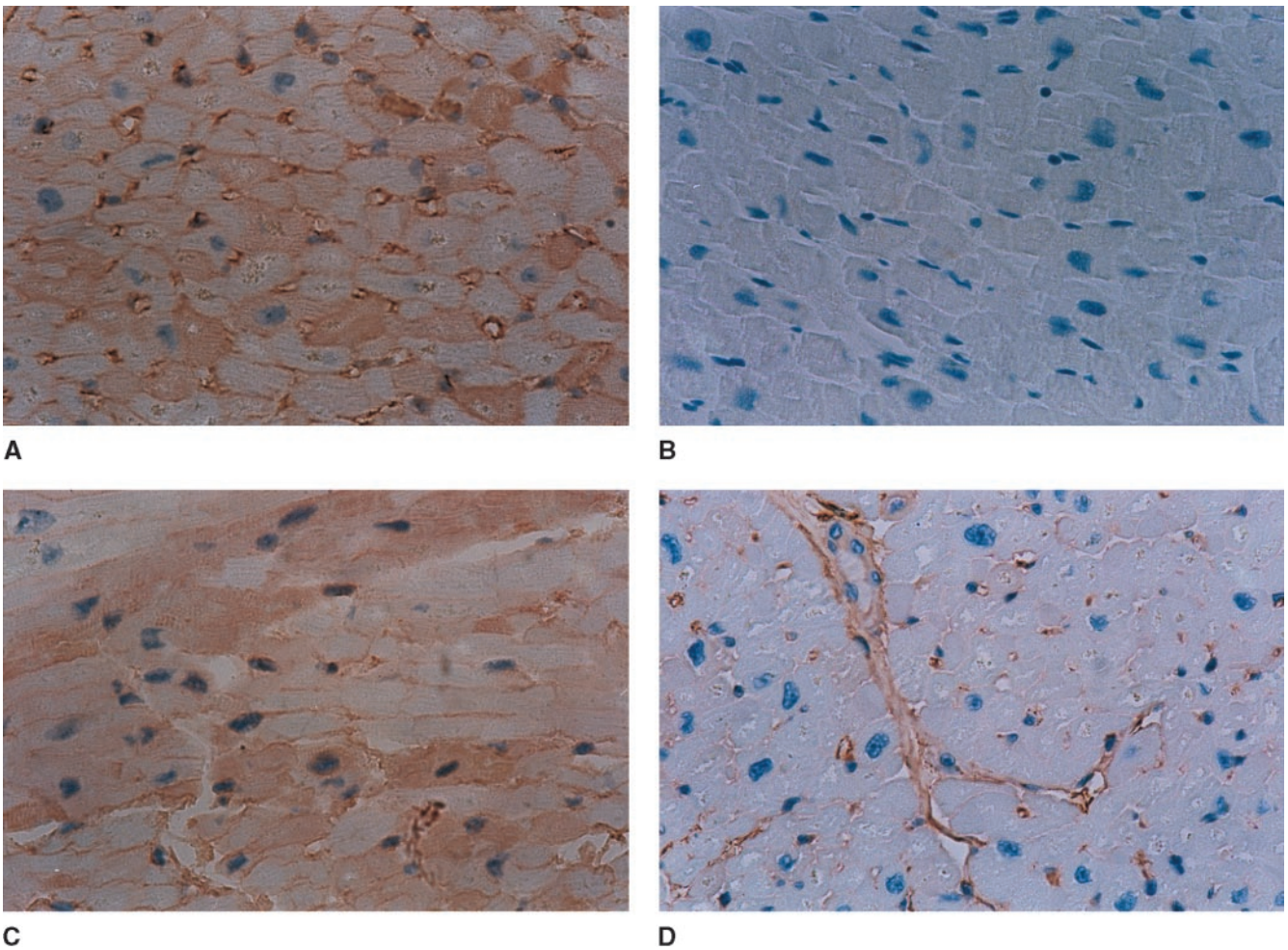

FIG. 4. *In situ* hybridization for D-glyceraldehyde-3-phosphate dehydrogenase, ET_A, and ET_B mRNA in myocardial sections of left ventricular tissue of human donors (magnifications ×400). (A) positive GAPDH mRNA signals in both myocytes and interstitial cells of left ventricular tissue and (B) RNAase treatment of the same specimen. (C) positive ET_A mRNA signals in both myocytes and non-myocyte cells of left ventricle. (D) positive ET_B mRNA signals in left ventricular interstitial cells.

secondly, and probably more important, the sensitivity of non-radioactive *in situ* hybridization might be insufficient to appreciate the small amount of mRNA for ET_B subtype expressed in cardiomyocytes. This different distribution of the two ET receptor subtypes between myocyte and non-myocyte in the left ventricle of human and pigs is similar to that reported in previous binding studies performed on human atrial [3] and rat ventricular myocytes and fibroblasts [4]. In the present study, we decided to investigate ET receptor binding only on freshly isolated, immediately used myocytes and not on cultured fibroblasts because of the possible changes in membrane receptors induced by cell passages in culture, as demonstrated for other seven-transmembrane domain receptors [24, 25].

The interest of the prevalent expression of ET_A on human ventricular cardiomyocytes stems from the observation that only ET_A receptors are functionally important in human heart [26], with the phospholipase C/inositol triphosphate/diacylglycerol system representing the major intracellular signaling pathway [6]. Furthermore, ET_A subtypes have different intracellular effects between atrial and ventricular cells because, as previously demonstrated in studies performed on cardiac membranes, ET_A receptors couple to IP formation and inhibition of adenilcyclase in atrial preparations [26], whereas in left ventricle they couple only IP formation [26]. Under our experimental conditions, the ^{125}I-ET-1 binding to human cardiomyocytes matched the characteristics of receptor binding such as saturability, rapidity, and specificity of the binding, and showed a binding affinity in the low nanomolar range. However, using kinetic analysis we estimated that in both human and pig hearts the affinities of ET-1 for ET_A receptors were about twice as high as those determined in saturation analysis. These minor differences could be related to the slow kinetic of ^{125}I-ET-1 dissociation (Fig. 1), since determination of the K_d by kinetic analysis provides a more accurate estimate of affinity than do Scatchard analysis and saturation binding when the binding kinetics are very slow [20]. Other investigators have also reported picomolar or higher affinities for ^{125}I-ET-1 binding to its receptors using kinetic analysis, and much lower affinity in saturation analysis [27–30]. Thus, the discrepancy between the two calculated K_d for the ^{125}I-ET-1 binding might indicate the effects of the slow equilibration time of the binding reaction.

A previous study performed using FR139317 reported a lower affinity and a higher density of ET_A cardiac receptors in pig heart in comparison to values obtained in adult humans, but this difference probably reflects the neonatal source of porcine tissue [11]. In our study, no major differences were detected between human and pig ET receptors on isolated cardiomyocytes. The close similarity between human and swine ET receptor binding properties seems to be particularly relevant, given that these animals are considered among the most appropriate donors for cardiac xenotransplantation [31]. Cyclosporine was reported to enhance ET-1 formation [32], and an increased cardiac mRNA expression for ET-1 appears to be colocalized to foci of fibroblast proliferation and myocardial fibrosis in humans following cardiac transplantation [33]. The human–swine similarity in ET-1 receptor binding could make possible the use of the same ET_A antagonists adopted for human use in recipients of swine cardiac xenotransplantation as well.

Differences, however, do seem to exist when the present findings are compared with those previously reported in rat. These pharmacological differences between human and rat regarding both the ET_B [11] and ET_A subtypes [9, 11] may be accounted for by the 7–9% and 12% species difference in the primary sequences of ET_A and ET_B receptors, respectively [9, 34, 35].

In conclusion, the present findings obtained in normal human and pig hearts indicate that cardiomyocytes express mainly ET_A but also a low percentage of ET_B receptors, and that no major differences exist between human and pig ET receptors.

References

1. Arai H, Hori S, Aramori I, Ohkubo H and Nakanishi S, Cloning and expression of cDNA encoding an endothelin receptor. *Nature* **348:** 730–732, 1990.
2. Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Katsutoshi G and Masaki T, Cloning of cDNA encoding a non-isopeptide-selective subtype of endothelin receptor. *Nature* **348:** 732–735, 1990.
3. Molenaar P, O'Reilly G, Sharkey A, Kuc RE, Harding DP, Plumpton C, Gresham A and Davenport AP, Characterization and localization of endothelin receptor subtypes in the human atrioventricular conducting system and myocardium. *Circ Res* **72:** 526–538, 1993.
4. Fareh J, Toujz RM, Shiffrin EL and Thibault G, Endothelin 1 and angiotensin II receptors in cells from rat hypertrophied heart. *Circ Res* **78:** 302–311, 1996.
5. Hilal-Dandan R, Villegas S, Gonzalez A and Brunton LL, The quasi-irreversible nature of endothelin binding and G protein-linked signaling in cardiac myocytes. *J Pharmacol Exp Ther* **281:** 267–273, 1997.
6. Rubany GM and Polokoff MA, Endothelins: Molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol Rev* **46:** 325–415, 1994.
7. Tsukahara H, Ende H, Magazine HI, Bahou WF and Goligorski MS, Molecular and functional characterization of the non-isopeptide-selective ET_B receptor in endothelial cells. *J Biol Chem* **269:** 21778–21785, 1994.
8. Takanashi M and Endoh M, Characterization of positive inotropic effect of endothelin on mammalian ventricular myocardium. *Am J Physiol* **261:** H611–H619, 1991.
9. Elshourbagy NA, Korman DR, Wu HL, Sylvester DR, Lee JA, Nuthalganti P, Bergsma CS, Kumar CS and Nambi P, Molecular characterization and regulation of human endothelin receptors. *J Biol Chem* **268:** 3873–3879, 1993.
10. Arai H, Nakao K, Takaya K, Hosoda K, Ogawa Y, Nakanishi S and Imura H, The human endothelin-B receptor gene: Structural organization and chromosomal assignment. *J Biol Chem* **268:** 3463–3470, 1993.
11. Peter MG and Davenport AP, Selectivity of [^{125}I]-PD151242 for human, rat and porcine endothelin ET_A receptors in the heart. *Br J Pharmacol* **114:** 297–302, 1995.
12. Yang HT, Zhu Y and Endoh M, Species-dependent differ-

ences in inotropic effects and phosphoinositide hydrolysis induced by endothelin-3 in mammalian ventricular myocardium. *Br J Pharmacol* **120:** 1497–1504, 1997.
13. Battler A, Scheinowitz M, Bor A, Hasdai D, Vered Z, Di Segni E, Varda-Bloom N, Nass D, Engelberg S and Eldar M, Intracoronary injection of basic fibroblast growth factor enhances angiogenesis in infarcted swine myocardium. *J Am Coll Cardiol* **22:** 2001–2006, 1993.
14. Battler A, Hasdai D, Goldberg I, Ohad D, Di Segni E, Bor A, Varda-Bloom N, Vered Z, Kornowski R, Lake M, Nass D and Savion N, Exogenous insulin-like growth factor II enhances post-infarction regional myocardial function in swine. *Eur Heart J* **16:** 1851–1859, 1995.
15. Fricker J, Baboon xenotransplant fails but patient improves. *Lancet* **347:** 457, 1996.
16. Stein PD, Hunt JT, Floyd DM, Moreland S, Dickenson KEJ, Mitchell C, Liu ECK and Webb ML, The discovery of sulfonamide endothelin antagonist and the development of the orally active ET_A antagonist 5-(dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalene sulfonamide. *J Med Chem* **37:** 329–331, 1994.
17. Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, Fukuroda T, Fukami T, Ozaki S, Nagase T, Nishikibe M and Yano M, Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc Natl Acad Sci USA* **91:** 4892–4896, 1994.
18. Human Experimentation. Code of ethics of the World Medical Association. *Br Med J* **ii:** 177–180, 1964.
19. Weiland GA and Molinoff PB, Quantitative analysis of drug–receptor interactions: I. Determination of kinetic and equilibrium properties. *Life Sci* **29:** 313–330, 1981.
20. Cheng YC and Prusoff WH, Relation between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzyme reaction. *Biochem Pharmacol* **22:** 3099–3109, 1973.
21. Modesti PA, Cecioni I, Colella A, Costoli C, Paniccia R and Neri Serneri GG, Binding kinetics and antiplatelet activities of picotamide, a TxA_2 receptor antagonist. *Br J Pharmacol* **112:** 81–86, 1994.
22. Modesti PA, Cecioni I, Migliorini A, Naldoni A, Costoli A, Vanni S and Neri Serneri GG, Increased renal endothelin formation is associated with sodium retention and increased free water clearance. *Am J Physiol* **275:** H1079–H1077, 1998.
23. Hosoda K, Nakao K, Arai H, Suga S, Ogawa Y, Mukoyama M, Shirakami G, Saito Y, Nakanishi S and Imura H, Cloning and expression of human endothelin-1 receptor cDNA. *FEBS Lett* **287:** 23–26, 1991.
24. Matsubara H, Kanasaki M, Murasawa S, Tsukaguchi Y, Nio Y and Inada M, Differential gene expression and regulation of angiotensin II receptor subtype in rat cardiac fibroblasts and cardiomyocytes in culture. *J Clin Invest* **93:** 1592–1601, 1994.
25. Neuß M, Regitz-Zagrosek V, Hildebrandt A and Fleck E, Isolation and characterization of human cardiac fibroblasts from explanted adult hearts. *Cell Tissue Res* **286:** 145–153, 1996.
26. Pönicke K, Volgelsang M, Heinroth M, Becker K, Zolk O, Bohm M, Zerkowski HR and Brodde OE, Endothelin receptors in the failing and nonfailing human heart. *Circulation* **97:** 744–751, 1998.
27. Waggoner WG, Genova SL and Rash VA, Kinetic analyses demonstrate that the equilibrium assumption does not apply to [^{125}I]-endothelin-1 binding data. *Life Sci* **51:** 1869–1876, 1992.
28. Leite MF, Page E and Ambler SK, Regulation of ANP secretion by endothelin-1 in cultured atrial myocytes: Desensitization and receptor subtype. *Am J Physiol* **267:** H2193–H2203, 1994.
29. Davenport AP, Kuc RE, Ashby MJ, Patt WC and Doherty AM, Characterization of [^{125}I]-PD164333, an ET_A selective non-peptide radiolabelled antagonist, in normal and diseased human tissues. *Br J Pharmacol* **123:** 223–230, 1998.
30. Desmarets J, Gresser O, Guedin D and Frein C, Interaction of endothelin-1 with cloned ETA receptors: Biochemical parameters and functional consequences. *Biochemistry* **35:** 14868–14875, 1996.
31. Dorling A, Riesbeck K, Warrens A and Lechler R, Clinical xenotransplantation of solid organs. *Lancet* **349:** 867–871, 1997.
32. Nayler WG, Gu XH, Casley DJ, Panagiotopoulos S, Liu J and Mottram PL, Cyclosporine increases endothelin-1 binding site density in cardiac cell membranes. *Biochem Biophys Res Commun* **163:** 1270–1274, 1989.
33. Giaid A, Saleh D, Yanagisawa M and Clarke Forbes RD, Endothelin immunoreactivity and mRNA in the transplanted human heart. *Transplantation* **59:** 1308–1313, 1995.
34. Adachi M, Yang YY, Furuichi Y and Miyamoto C, Cloning and characterization of cDNA encoding human A-type endothelin receptor. *Biochem Biophys Res Commun* **180:** 1265–1272, 1991.
35. Ogawa Y, Nakao K and Arai H, Molecular cloning of a non-isopeptide-selective human endothelin receptor. *Biochem Biophys Res Commun* **178:** 248–255, 1991.