Impaired Response to ET_B Receptor Stimulation in Heart Failure: Functional Evidence of Endocardial Endothelial Dysfunction?

CARMEN BRÁS-SILVA, ANA PATRÍCIA FONTES-SOUSA, CLÁUDIA MOURA, JOSÉ CARLOS AREIAS, AND ADELINO F. LEITE-MOREIRA[1]

Department of Physiology, Faculty of Medicine, University of Porto, Portugal

Inotropic effects of selective ET_B receptor stimulation depend on the functional integrity of the endocardial endothelium (EE), which is negative when it is intact and positive when it is damaged. These results have been attributed to the existence of two subtypes of ET_B receptors in the heart: (i) ET_{B1}, located on the EE, decreases inotropy; (ii) ET_{B2}, located on myocardial cells, increases inotropy. In the present study we investigated the functional integrity of the EE in a heart failure (HF) model (doxorubicin-induced cardiomyopathy) by evaluating the contractile response to ET_{B1} receptor stimulation. New Zealand White rabbits were treated with doxorubicin (DOX-HF, 1 mg/kg, iv, twice weekly for 8 weeks) or with saline. Contractile effects of increasing doses of a selective agonist of endothelial ET_B receptors, IRL-1620 (10^{-9} to 10^{-6} *M*), were studied in papillary muscles (Krebs-Ringer: 1.8 m*M* $CaCl_2$, 35°C) from control ($n = 10$) and DOX-HF rabbits ($n = 7$). Isotonic and isometric twitches were recorded and analyzed. Reported parameters included active tension (AT) and maximum velocities of tension rise (dT/dt_{max}) and decline (dT/dt_{min}). On echocardiography, DOX-HF rabbits had increased left ventricular (LV) end-diastolic and end-systolic diameters and reduced ejection fraction (52% ± 2% vs. 61% ± 1%). Contrary to control papillary muscles, DOX-HF muscles showed a steady decrease in contractility between 1 and 4 Hz. In the control group, IRL-1620 induced dose-dependent negative inotropic and lusitropic effects that decreased at 10^{-6} *M*: 26% ± 3%, AT; 17% ± 3%, dT/dt_{max}; and 16% ± 5%, dT/dt_{min}. In the DOX-HF group, these effects were significantly reduced. At the same concentration, IRL-1620 decreased AT (8% ± 3%) and dT/dt_{max} (8% ± 3%), without significantly affecting dT/dt_{min}. This study showed an impaired response to endothelial ET_B receptor stimulation, providing for the first time strong evidence of the occurrence of EE dysfunction in the failing heart and further highlighting the potential use of ET_B receptor stimulation as a marker of EE function. Exp Biol Med 231:893–898, 2006

Key words: heart; endothelin; endothelial function; ET_B receptors; contractile function; heart failure

Introduction

The discovery in 1988 of endothelin-1 (ET-1), one of the most potent endogenous vasoconstrictor peptides, by Yanagisawa and colleagues (1) represented a landmark in the field of cardiovascular research. Since its discovery, a great deal of effort has been made toward gaining a better understanding of the key roles (developmental, physiological, and pathological) played by this peptide, particularly with regard to the cardiovascular system.

ET-1 exerts its actions mainly through two types of receptors, the so-called type A (ET_A) and type B (ET_B) receptors. Both are G protein–coupled transmembrane proteins, with different molecular and pharmacologic characteristics and functions based on their location (2–4).

ET_A receptor stimulation elicits vasoconstriction (5) and mitogenesis (6) and increases inotropism (7, 8) and myocardial distensibility in conditions of cardiac overload (9). ET_B receptor activation promotes vasodilatation mediated by nitric oxide and prostacyclin (10) release and has growth-inhibitory effects (11) associated with apoptosis (12). In addition, ET_B receptors play a determinant role in the clearance of circulating ET-1 (13).

There is increasing experimental and clinical evidence in support of an important role of ET-1 in the pathophysiology of heart failure (HF) (14). The endothelin system is activated in patients with chronic HF. Plasma big ET-1 and ET-1 concentrations have been correlated with clinical and hemodynamic measures of severity in patients with HF and inversely with prognosis (14, 15).

This work was supported by grants from the Portuguese Foundation for Science and Technology (POCI/SAU-CBO/47519/2002 and POCI/SAU-FCT/60803/2004) through the Cardiovascular Research & Development Unit (FCT 51/94).

[1] To whom correspondence should be addressed at Department of Physiology, Faculty of Medicine, Alameda Professor Hernâni Monteiro, 4200–319 Porto, Portugal. E-mail: amoreira@med.up.pt

Received September 29, 2005.
Accepted November 10, 2005.

1535-3702/06/2316-0893$15.00

Doxorubicin is a commonly used chemotherapeutic agent that is associated with the development of dose-dependent cardiomyopathy and irreversible and progressive HF characterized by bilateral enlargement, thinning of the ventricular wall, and reduction of the ejection fraction. Doxorubicin-induced HF (DOX-HF) has been used in different animal species to study the pathophysiologic mechanisms and to evaluate different treatment modalities for HF (16).

It was recently shown that the inotropic effect of selective ET_B receptor stimulation depends on the functional integrity of the endocardial endothelium (EE), which is negative when it is intact and positive when it is damaged. These results have been attributed to the existence of two subtypes of ET_B receptors in the heart: ET_{B1}, which is located on the EE and decreases inotropy, and ET_{B2}, which is located on myocardial cells and increases inotropy (17). The differential effects of ET_B stimulation in the presence and absence of an intact EE indicate that the analysis of such effects might be used as an experimental tool to test the functional integrity of the EE (17). In this context, the main goal of the present study was to investigate the functional integrity of the EE in an HF model (DOX-HF) by evaluating the contractile response to ET_{B1} stimulation.

Materials and Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, Revised 1996).

HF Model. A well-documented regimen was used for the induction of HF due to doxorubicin toxicity (DOX-HF) (18). Adult male New Zealand White rabbits (*Oryctolagus cuniculus*; 2.0–3.0 kg) received doxorubicin *via* a marginal ear vein by bolus injection (1 mg/kg) twice weekly for 8 weeks. Control rabbits received the vehicle (0.9% saline) in equivolumetric doses over the same period. The progression of cardiac dysfunction was monitored echocardiographically to estimate morphological and functional alterations during the development of HF.

Echocardiographic Evaluation. All animals were evaluated by echocardiography at the beginning of the study and then every 2 weeks during the study. Echocardiographic examination was performed with the rabbits lightly anesthetized with an intramuscular combination of ketamine (15 mg/kg) and medetomidine (0.15 mg/kg), and rabbits were allowed to breathe spontaneously. The animal was placed prone on a table with an area removed so that the ultrasound probe could be brought from below and placed on a shaved area of the anterior chest wall. The echocardiograms were obtained using a 5-MHz transducer (Aloka Color Doppler SSD-2200 echocardiograph; Aloka S.A., Tokyo, Japan), and the exam was performed from the right paraesternal position. Three representative cycles were measured and averaged for each rabbit. Parameters analyzed were heart rate, anterior and posterior end-diastolic and end-systolic wall thickness, left ventricular end-systolic and end-diastolic diameters (ESD and EDD, respectively), fractional shortening (FS; FS= [EDD − ESD]/EDD), and ejection fraction.

Papillary Muscle Studies. *Experimental Preparation.* The study was performed in isolated right papillary muscles ($n = 31$) from the control and DOX-HF groups 1 week after the last drug or saline administration. Rabbits were anesthetized with intravenous pentobarbital sodium salt (25 mg/kg). A left thoracotomy was performed, and beating hearts were quickly excised and immersed in modified Krebs-Ringer (KR) solution (composition in m*M*: NaCl, 98; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 2.4; KH_2PO_4, 1.2; glucose, 4.5; $CaCl_2 \cdot 2H_2O$, 1.8; $NaHCO_3$, 17; $C_3H_3NaO_3$, 15; CH_3COONa, 5; atenolol, 0.02) at 35°C with cardioplegic 2,3-butanedione monoxime (BDM; 3%) and 5% of newborn calf serum and gassed with 95% O_2/5% CO_2, to obtain a pH between 7.38 and 7.42.

After dissection, papillary muscles (length: 4.2 ± 0.3 mm; weight: 2.9 ± 0.3 mg; preload: 4.3 ± 0.3 mN) were mounted vertically in a 10-ml Plexiglas organ bath containing the above-described KR solution and were attached to an electromagnetic length-tension transducer (University of Antwerp, Belgium). Preload was estimated according to muscle dimensions, and the electrical stimulus (0.6 Hz) was set at 10% above threshold. Twenty minutes later, bathing solutions were replaced by corresponding KR solutions without BDM. During the next 2 hrs, muscles were stabilized. Bathing solutions were then replaced by corresponding KR solutions without calf serum, and L_{max} was calculated. Protocols were initiated after obtaining two similar isotonic and isometric control twitches separated by a 10-min interval.

Experimental Protocols. Effects of increasing doses of a selective agonist of endothelial ET_B receptors, IRL-1620 (10^{-9} to 10^{-6} *M*), were studied in papillary muscles from the control ($n = 10$) and DOX-HF ($n = 7$) groups.

In another set of papillary muscles from control ($n = 7$) and DOX-HF ($n = 7$) groups, isometric contractility-frequency relationships were obtained by plotting the maximum velocity of tension rise against the frequency of contraction. In summary, after an initial period of contraction at 0.6 Hz, the frequency of stimulation was stepped up at 3-min intervals to 1 Hz, 2 Hz, 3 Hz, and 4 Hz. Drugs were obtained from Sigma Chemical Company (St. Louis, MO).

Data Analysis. Isotonic and isometric twitches were recorded and analyzed. Selected parameters included active tension (AT, mN/mm^2); maximum velocity of tension rise (dT/dt_{max}, $mN/mm^2/sec$); maximum velocity of tension decline (dT/dt_{min}, $mN/mm^2/sec$); peak isotonic shortening (PS, $\%L_{max}$); maximum velocity of shortening (dL/dt_{max}, L_{max}/sec), maximum velocity of lengthening (dL/dt_{min}, L_{max}/sec); and time to half relaxation (tHR, msec).

Only data obtained from isometric twitches will be

Table 1. Mean Values of the Contractile Parameters in Papillary Muscles from the Control and Doxorubicin-Induced Heart Failure (DOX-HF) Groups[a]

Contractile parameter	Control group ($n = 17$)	DOX-HF group ($n = 14$)
AT (mN/mm^2)	25.3 ± 3.0	25.8 ± 2.4
dT/dt_{max} ($mN/mm^2/sec$)	175.4 ± 18.5	173.2 ± 15.7
dT/dt_{min} ($mN/mm^2/sec$)	−137.0 ± 15.9	−132.6 ± 13.2
PS ($\%L_{max}$)	12.0 ± 0.1	12.0 ± 0.1
dL/dt_{max} (L_{max}/sec)	0.9 ± 0.01	0.8 ± 0.07
dL/dt_{min} (L_{max}/sec)	−3.0 ± 0.4	−2.7 ± 0.4
tHR (msec)	377.0 ± 14.6	407.1 ± 21.1

[a] Values are means ± SEM. EE, endocardial endothelium; AT, active tension; dT/dt_{max}, maximum velocity of tension rise; dT/dt_{min}, maximum velocity of tension decline; PS, peak isotonic shortening; dL/dt_{max}, maximum velocity of shortening; dL/dt_{min}, maximum velocity of lengthening; tHR, time to half relaxation.

described, as the analysis of isotonic twitches yielded globally similar results. In the various protocols, results are given as percent changes from baseline. For the parameters that are expressed as negative values (e.g., dT/dt_{min}), such percent change refers to the absolute values.

Statistical Methods. Values are means ± SEM. Echocardiographic data of doxorubicin-treated animals at the beginning and at the end of the study were compared with a paired *t* test. Baseline performance of papillary muscles from control and doxorubicin-treated rabbits was compared with an unpaired *t* test. Effects of increasing concentrations of IRL-1620 and of increasing stimulation frequencies of papillary muscles from control and doxorubicin-treated rabbits were analyzed with a repeated-measures two-way analysis of variance. When significant differences were detected, the Tukey's *post hoc* test was selected to perform multiple comparisons; $P < 0.05$ was accepted as significant.

Results

Mean values of the contractile parameters in papillary muscles from the control group ($n = 17$) and from the DOX-HF group ($n = 14$) are shown in Table 1. Although baseline performance of rabbit papillary muscles was similar in all experimental protocols, contractility of papillary muscles from the control group did not significantly decline with increasing frequency (between 1 Hz and 4 Hz), whereas the papillary muscles from the DOX-HF rabbits showed a decrease in contractility with increasing frequency, indicative of contractile dysfunction and a reduced contractile reserve (Fig. 1). Additionally, in the DOX-HF group, the echocardiographic evaluation demonstrated a progressive increase of end-diastolic (from 14.3 ± 0.8 mm to 15.6 ± 0.4 mm) and end-systolic (from 10.4 ± 0.3 mm to 11.7 ± 0.4 mm) short-axis diameters and a reduction in fractional shortening (from 30% ± 1% to 24% ± 1%) and ejection fraction (from 61% ± 1% to 52% ± 2%) of the left ventricle, consistent with the presence of dilated cardiomyopathy and HF.

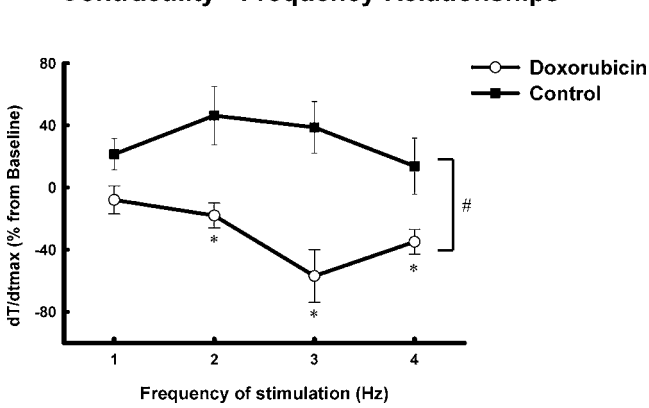

Figure 1. Contractile response of rabbit papillary muscles from the control group ($n = 7$) and from the doxorubicin-induced heart failure (DOX-HF) group ($n = 7$) to steady increases in stimulation frequency. Contractility-frequency relationships were then obtained by plotting maximum velocity of tension rise against frequency of contraction. Control muscles showed a steady increase in contractility between 1 Hz and 4 Hz, whereas in the DOX-HF group, muscles responded in the opposite way. $P < 0.05$: *, versus baseline; #, versus control.

Myocardial Effects of Selective ET_B Receptor Stimulation by IRL-1620. Figures 2 and 3 illustrate the effects of selective stimulation of the endothelial ET_B receptor with the agonist IRL-1620 in the various experimental conditions. In the control group, IRL-1620 induced dose-dependent negative inotropic and lusitropic effects. At 10^{-6} *M*, it significantly decreased AT (26% ± 3%), dT/dt_{max} (17% ± 3%), dT/dt_{min} (16% ± 5%), and tHR (11% ± 2%). In the DOX-HF group, these effects were significantly reduced. At the same concentration IRL-1620 decreased AT (8% ± 3%) and dT/dt_{max} (8% ± 4%), without significantly affecting dT/dt_{min} or tHR (Figs. 2 and 3).

Discussion

The present study showed that in the presence of HF induced by doxorubicin (DOX-HF), the myocardial response to selective endothelial ET_B receptor stimulation is impaired. Thus, in healthy animals (control group), IRL-1620 induced significant negative inotropic and lusitropic effects that were clearly reduced in papillary muscles from the failing hearts.

The progression of cardiac dysfunction was monitored echocardiographically to estimate morphologic and functional alterations during the development of HF. In addition, as contractile dysfunction in papillary muscles is most often not evident from changes in baseline performance of muscles that are contracting at low stimulating frequencies, but rather is evident based on an impaired response to increased frequencies (19), contractility-frequency relationships were performed. We found that although baseline

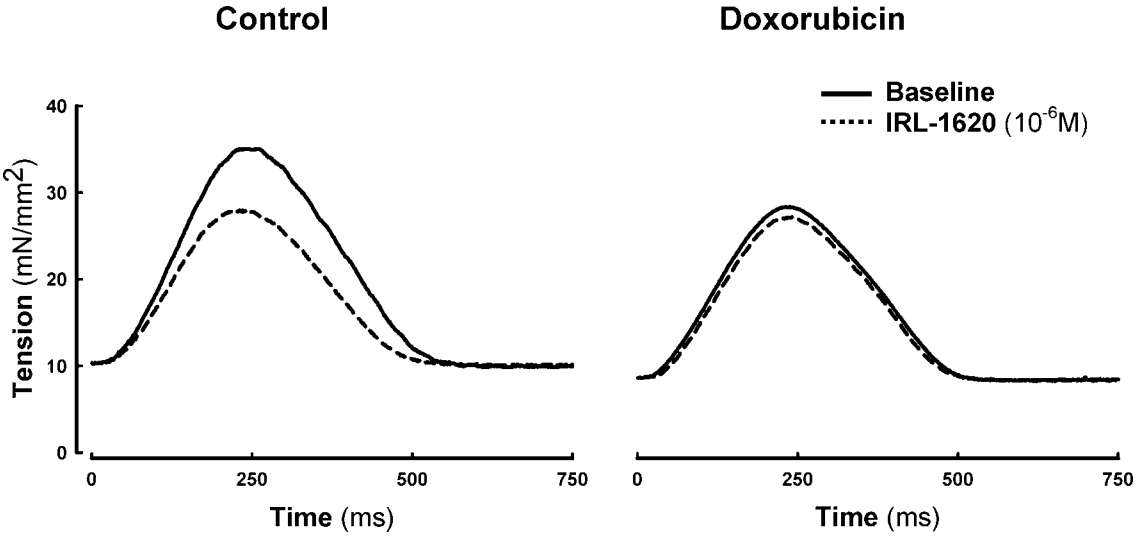

Figure 2. Representative isometric twitches performed in rabbit papillary muscles from the control group and from the doxorubicin-induced heart failure (DOX-HF) group, showing the effects of selective endothelial ET_B receptor stimulation by IRL-1620 (10^{-6} *M*). IRL-1620 induced negative inotropic and lusitropic effects in the representative twitch from the control group, effects that were clearly reduced in the example from the DOX-HF group.

performance of normal and DOX-HF muscles was similar, contrary to the former, the latter showed decreased contractility to increased frequencies, indicating contractile dysfunction and reduced contractile reserve.

The role of cardiac endothelium (EE and myocardial capillary endothelium) in HF has only recently been addressed. Typical morphologic EE cellular lesions have now been described in conditions of ventricular volume (20) or pressure (21, 22) overload. Experimental *in vitro* studies have demonstrated selective damage of the EE after exposure to high concentrations of a number of neurohormones and stressors known to be pathogenic risk factors *in vivo*, such as high plasma levels of catecholamines, angiotensin, atrial natriuretic peptide, serotonin, vasopressin, ox–low-density lipoproteins, homocysteine, cholic acid, and eosinophils. These lesions were accompanied by profound changes in the mechanical performance of the subjacent myocardium. Most cardiovascular risk factors known to be pathogenic for other vascular endothelial cells appear to also affect EE as an early target, contributing to the etiology and progression of cardiac failure (23). The association of such EE lesions with these conditions indicates that they might contribute causally to cardiac failure, but experimental evidence that they do so has been missing.

Until recently, a major limitation for the evaluation of EE dysfunction was the nonexistence of a functional marker, like acetylcholine for the vascular endothelium. We have recently gathered evidence that the response to selective ET_B receptor stimulation might be used as such a marker. In fact, similar to acetylcholine in the vasculature, myocardial effects of ET_B receptor stimulation depend on the presence or absence of a functional EE. When the EE is intact, endothelial ET_B receptor stimulation promotes negative inotropic and lusitropic effects that are mediated by nitric oxide and prostaglandins. On the contrary, when the EE is damaged, myocardial ET_B receptor stimulation induces positive inotropic and lusitropic effects (17). Therefore, if we use a selective endothelial ET_B receptor stimulator, we shall obtain negative inotropic and lusitropic effects when the EE is intact and no significant effects when the EE is damaged, as was previously shown (17). In this setting, the present study, having shown that papillary muscles from failing hearts had a blunted response to selective endothelial ET_B receptor stimulation, provides strong evidence in favor of the presence of EE dysfunction in the HF model used. Thus, as is the case with vascular endothelial dysfunction, it seems that cardiac endothelial dysfunction is present and/or may contribute to HF progression.

Although some concern can be raised with regard to the selectivity of IRL-1620 at higher concentrations, especially at 10^{-6} *M*, the results of this and other studies (17, 24) are not in favor of such a possibility. In fact, if this was the case, IRL-1620 (10^{-6} *M*) should increase contractility of papillary muscles devoid of an intact EE. We showed, however, that in these circumstances, IRL-1620 does not have any significant effects on muscular performance (17, 24).

Doxorubicin is an antineoplastic antibiotic widely used in the treatment of a variety of cancers, and its clinical use is limited as a result of a severe, dose-dependent cardiotoxicity (16, 18). In this context, our findings might also be relevant to better understand the pathophysiology of DOX-induced cardiomyopathy so that we can develop efficient protective and/or therapeutic strategies in patients treated with this chemotherapeutic agent.

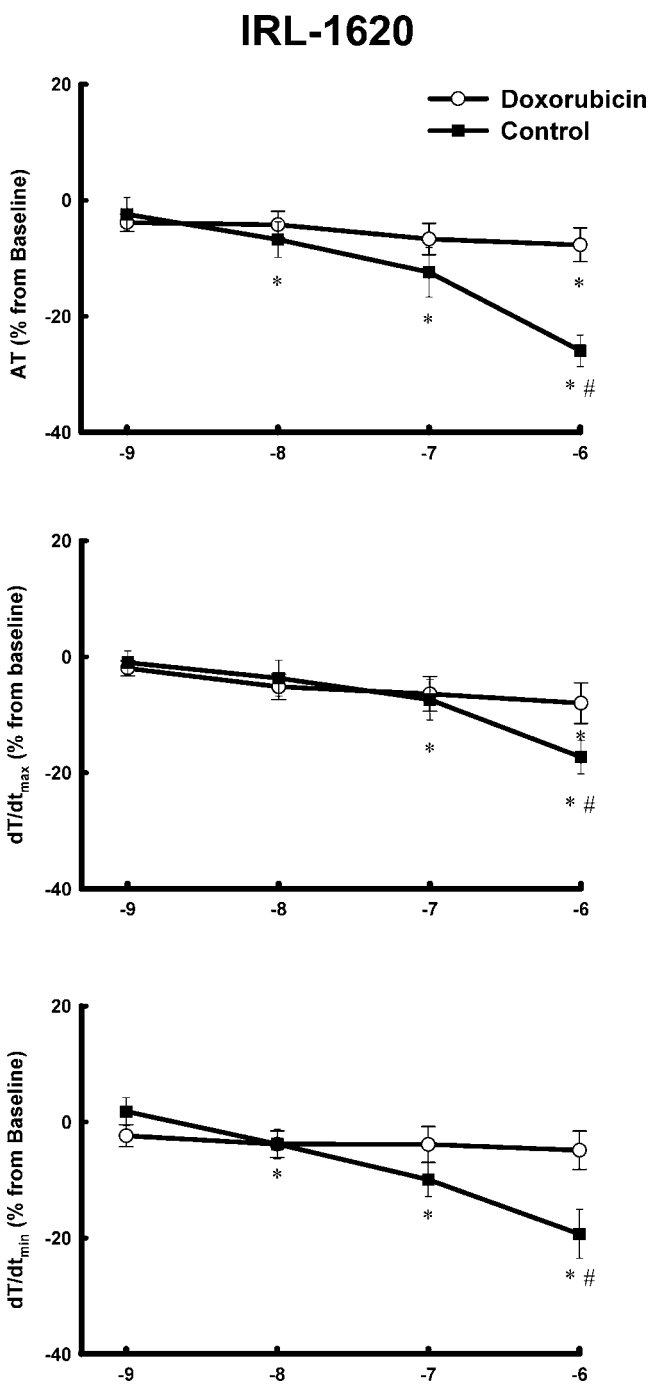

Figure 3. Concentration-response curves for the effect of selective endothelial ET_B receptor stimulation by IRL-1620 on contractile parameters in the various experimental conditions: control group (full squares, $n = 10$) or doxorubicin-induced heart failure (DOX-HF) group (open circles, $n = 7$). AT, active tension, top panel; dT/dt_{max}, maximum velocity of tension rise, middle panel; and dT/dt_{min}, maximum velocity of tension decline, bottom panel. Mean ± SEM; % baseline. $P < 0.05$: *, versus baseline; #, versus control.

This study showed an impaired response to endothelial ET_B receptor stimulation, indicating the presence of EE dysfunction in the experimental model of HF induced by doxorubicin and reinforcing the importance of ET_{B1} receptors as functional markers of endothelial integrity. Additionally, these results might be relevant for a better understanding of the role of EE in the pathophysiology of HF.

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411–415, 1988.
2. Arai H, Hori H, Aramori A, Ohkubo H, Nakanishi S. Cloning and expression of cDNA encoding an endothelin receptor. Nature 348:730–732, 1990.
3. Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K, Masaki T. Cloning of a cDNA encoding a non-iso peptide selective subtype of the endothelin receptor. Nature 348:732–735, 1990.
4. Sudjarwo SA, Hori M, Tanaki T, Matsuda Y, Okada T, Karaki H. Subtypes of ETA and ETB receptors mediating venous smooth muscle contraction. Biochem Biophys Res Commun 200:627–633, 1994.
5. Marsault R, Feolde E, Frelin C. Receptor externalisation determines sustained contractile responses to endothelin-1 in the rat aorta. Am J Physiol Cell Physiol 264:C687–C693, 1993.
6. Ohlstein EH, Arleth A, Bryan H, Elliot JD, Sung CP. The selective endothelin ETA receptor antagonist BQ123 antagonizes endothelin-1–mediated mitogenesis. Eur J Pharmacol 225:347–350, 1992.
7. Ishikawa T, Li LM, Shinmi O, Kimura S, Yanagisawa M, Goto K, Masaki T. Characteristics of binding of endothelin-1 and endothelin-3 to rat hearts. Developmental changes in mechanical responses and receptor subtypes. Circ Res 69:918–926, 1991.
8. Kelso EJ, McDermott BJ, Silke B, Spiers JP. Endothelin A receptor subtype mediates endothelin-induced contractility in left ventricular cardiomyocytes isolated from rabbit myocardium. J Pharmacol Exp Ther 294:1047–1052, 2000.
9. Leite-Moreira AF, Bras-Silva C, Pedrosa CA, Rocha-Sousa AA. ET-1 increases distensibility of acutely loaded myocardium: a novel ETA and Na+/H+ exchanger-mediated effect. Am J Physiol Heart Circ Physiol 284:H1332–H1339, 2003.
10. De Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, Vane JR. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by release of prostacyclin and endothelium-derived relaxation factor. Proc Natl Acad Sci U S A 85:9797–9800, 1988.
11. Mallat A, Fouassier L, Preaux AM, Gal CS, Raufaste D, Rosenbaum J, Dhumeaux D, Jouneaux C, Mavier P, Lotersztajn S. Growth inhibitory properties of endothelin-1 in human hepatic myofibroblastic Ito cells. An endothelin B receptor-mediated pathway. J Clin Invest 96:42–49, 1995.
12. Okazawa M, Shiraki T, Ninomiya H, Kobayashi S, Masaki T. Endothelin induced apoptosis of A375 human melanoma cells. J Biol Chem 267:12584–12592, 1998.
13. D'Orleans-Juste P, Labonte J, Bkaily G, Choufani S, Plante M, Honore JC. Function of the endothelin(B) receptor in cardiovascular physiology and pathophysiology. Pharmacol Ther 95:221–238, 2002.
14. Attina T, Camidge R, Newby DE, Webb DJ. Endothelin antagonism in pulmonary hypertension, heart failure, and beyond. Heart 91:825–831, 2005.
15. Brunner F, Bras-Silva C, Cerdeira AS, Leite-Moreira AF. Cardiovascular endothelins: essential regulators of cardiovascular homeostasis. Pharmacol Ther, 2006 Jan 31; [Epub ahead of print, doi 10.1016/j.pharmthera.2005.11.001]. In press.
16. Monnet E, Chachques JC. Animal models of heart failure: what is new? Ann Thorac Surg 79:1445–1453, 2005.
17. Leite-Moreira A, Brás-Silva C. Inotropic effects of ETB receptor stimulation and their modulation by endocardial endothelium, NO, and prostaglandins. Am J Physiol Heart Circ Physiol 287:H1194–H1199, 2004.

18. Arnolda L, McGrath B, Cocks M, Sumithran E, Johnston C. Adriamycin cardiomyopathy in the rabbit: an animal model of low output cardiac failure with activation of vasoconstrictor mechanisms. Cardiovasc Res 19:378–382, 1985.
19. Endoh M. Force-frequency relationship in intact mammalian ventricular myocardium: physiological and pathophysiological relevance. Eur J Pharmacol 500:73–86, 2004.
20. Masuda H, Kawamura K, Tohda K, Shozawa T, Sageshima M, Honma M. Endocardium of the left ventricle in volume-loaded canine heart. A histological and ultrastructural study. Acta Pathol Jpn 39:111–120, 1989.
21. Chu GX, Ling Q, Guo ZG. Effects of endocardial endothelium in myocardial mechanics of hypertrophied myocardium of rats. Acta Pharmacol Sin 16:352–356, 1995.
22. Smiley LM, Tyagi SC. Endocardial endothelium injury leading to fibrosis is one of the causes of cardiac failure in spontaneously hypertensive rats. Circulation 100:I-224–I-225, 1999.
23. Brutsaert DL. Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. Physiol Rev 83:59–115, 2003.
24. Brás-Silva C, Leite-Moreira AF. Obligatory role of the endocardial endothelium in the increase of myocardial distensibility induced by endothelin-1. Exp Biol Med 231:876–881, 2006.