Nitric oxide and prostaglandins – important players in endothelin-1 induced myocardial distensibility

Carmen Brás-Silva, Daniela Monteiro-Sousa, Ana Joana Duarte, Miguel Guerra, Ana Patrícia Fontes-Sousa, Cláudia Moura, José Carlos Areias, Adelino F. Leite-Moreira

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

Running Title: NO and prostaglandins modulate diastolic ET-1 effects

Address for correspondence:

Professor Adelino F. Leite-Moreira

Department of Physiology, Faculty of Medicine

Alameda Professor Hernâni Monteiro

4200-319 Porto, PORTUGAL

Tel: +/351/22/551.36.44

Fax: +/351/22/551.36.46

Email: amoreira@med.up.pt

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SUMMARY

This study investigated whether endothelin (ET)-1-induced increase in myocardial distensibility is preserved in heart failure (HF) and modulated by nitric oxide (NO) and prostaglandins.

New Zealand white rabbits were treated with Doxorubicin (1mg/kg, intravenously twice a weekly for 8 weeks, DOX-HF Group) or saline (Control Group). Effects of ET-1 (0.1, 1, 10nM) were tested in papillary muscles from the DOX-HF group and the Control Group in the presence of: (i) intact endocardial endothelium (EE); (ii) damaged EE; (iii) N^G-Nitro-L-Arginine (L-NNA; NO synthase inhibitor), and (iv) Indomethacin (INDO; cyclooxigenase inhibitor).

In the presence of an intact EE, ET-1 promoted concentration-dependent positive inotropic and lusitropic effects that were maintained after damaging the EE, in the presence of L-NNA or INDO and in the DOX-HF Group. ET-1 reduced resting tension at the end of the isometric twitch (increased diastolic distensibility) by 3.2±1.3%, 6.0±1.6% and 8.8±2.7% (at 0.1, 1 and 10nM, respectively), in muscles with intact EE, effect that was completely abolished after damaging EE, in the presence of L-NNA or INDO or in the DOX-HF Group.

This study demonstrated that the increase in myocardial distensibility induced by ET-1 is absent in HF and is dependent of NO and prostaglandin release.

Key words: endothelin; endothelium; heart failure; diastolic properties; myocardial distensibility.

INTRODUCTION

The discovery in 1988 of endothelin (ET)-1, one of the most potent endogenous vasoconstrictor peptides, by Yanagisawa and colleagues (1988) 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, where the components of the endothelin system are widely expressed, namely in vascular and endocardial endothelium, smooth muscle cells and cardiomyocytes (Brunner *et al*. 2006). ET-1 acts in two main subtypes of G-protein coupled receptors (ET_A and ET_B) and has mainly local autocrine and paracrine actions, since it is released abluminally and has a short half-life. In heart failure (HF) the plasmatic, salivary and tissue levels of ET-1 are increased and are positively related with the stage of the disease and negatively with its prognosis (Attina *et al*. 2005). ET_A receptors mediate vasoconstriction, mitogenesis and positive inotropism. ET_B receptor activation promotes mainly vasodilatation and has growth inhibitory effects associated with apoptosis. These receptors also mediate the pulmonary clearance of circulating ET-1 and the reuptake of ET-1 by endothelial cells. In the heart (Leite-Moreira and Bras-Silva 2004) and in the vasculature (Endoh *et al*. 1998) it is possible to further subclassify the ET_B receptors in ET_{B1} receptors, located on the vascular and endocardial endothelium and responsible for vasodilatation and negative inotropism, and ET_{B2} receptors, located on vascular muscular and myocardial cells and responsible for vasoconstriction and positive inotropism, respectively.

Unlike the well known role of chronically elevated ET-1 levels in progression to cardiac fibrosis and ventricular remodelling, the acute diastolic effects of ET-1 in the failing myocardium remain less explored. We have previously reported, in healthy animals, that ET-1 acutely decreases

myocardial stiffness in conditions of cardiac overload (Leite-Moreira *et al*. 2003). Although mediated by ET_A receptor stimulation (Leite-Moreira *et al*. 2003), this effect requires an intact endocardial endothelium (EE) and active endothelial ET_{B1} receptors (Bras-Silva and Leite-Moreira 2006). This is in agreement with the growing experimental evidence for a paracrine regulation of cardiac systolic and diastolic performance by endocardic endothelial cells that is analogous to vascular endothelial control of vascular tone (Brutsaert 2003). Until recently, a major limitation for the evaluation of EE dysfunction was the non-existence 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 and using this approach we documented endocardial endothelial dysfunction in an experimental model of HF, Doxorubicin-induced HF (Bras-Silva *et al.* 2006).

In this context, the present study was conducted in order to investigate whether the diastolic effects of ET-1 were preserved in HF, and whether they are dependent of two of the most important endothelial mediators, nitric oxide and prostaglandins.

METHODS

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication Nº 85-23, Revised 1996). The study was performed in New-Zealand White rabbits (*Oryctolagus cuniculus*; n=37; 1.8-3.0Kg)

Heart Failure Model

A well documented regimen was used for the induction of HF due to Doxorubicin toxicity (DOX-HF) (Arnolda *et al.* 1985). Adult male New Zealand White rabbits received Doxorubicin (DOX) via a marginal ear vein by bolus injection (1 mg/kg) twice weekly for 8 weeks (n=16) followed by a washout period of 1 week. Control rabbits (n=21) received the vehicle (0.9% saline)

in equivolumetric doses over the same period. Echocardiographic evaluation of all the animals was used to monitor left ventricular dilatation and dysfunction during the development of HF. In two subgroups of Control and DOX-HF hemodynamic evaluation was also performed. The experimental protocols were carried out in the isolated papillary muscle model.

Echocardiographic evaluation

All animals were evaluated by echocardiography at the beginning and every two weeks during the administration of DOX or vehicle. Echocardiographic examination was performed as previously described (Fontes-Sousa *et al.* 2006). Briefly, the rabbits were lightly anesthetized with an intramuscular combination of ketamine hydrochloride (2 mg/kg) and medetomidine hydrochloride (0.15 mg/kg), being allowed to breath spontaneously. The animals were 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 7.5MHz transducer (Vivid 3 General Electrics echocardiograph, Portugal) and the exam was performed from the right paraesternal short-axis view. Two-dimensional guided M-mode tracings were made just below the mitral valve at the level of the papillary muscles for measurements of the left ventricular internal diameter and left ventricular free wall in diastole and systole. Three representative cycles were measured and averaged for each rabbit at each time point. Analysed parameters 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].

Hemodynamic Assessment

The instrumentation of the animals for hemodynamic studies was performed one week after the last administration of vehicle (n=6) or DOX (n=9), respectively, as previously described (Leite-Moreira *et al*. 1999, Leite-Moreira and Correia-Pinto 2001). In summary, animals were

premedicated with ketamine hydrochloride (50 mg kg^{-1} *i.m.*) and xylazine hydrochloride (5 mg kg^{-1} *i.m.*). An auricular vein was cannulated, and a prewarmed solution containing 20 meq KCl and 40 meq $NaHCO_3$ in 500 ml of 0.9% NaCl was administrated to compensate for perioperative fluid losses. A tracheostomy was performed, and mechanical ventilation was initiated (Harvard Small Animal Ventilator, model 683), delivering oxygen enriched air. Respiratory rate and tidal volume were adjusted to keep arterial blood gases and pH within physiological limits. Anaesthesia was maintained with ketamine hydrochloride (33 ml kg h^{-1} *i.m.*) and pentobarbital sodium (12.5 mg kg^{-1} *i.v.* before opening the chest, and then 2.5 mg kg^{-1} *i.v.* as needed). A 20-gauge catheter was inserted in the right femoral artery and connected to a pressure transducer to monitor heart rate and arterial pressure, and to obtain samples for blood gas analysis. The heart was exposed by a median sternotomy, and the pericardium was widely opened. Transient aortic constrictions were performed by abruptly occluding the aorta with a silk suture placed around the ascending aorta during the diastole separating two heartbeats. This was achieved by pushing a plastic tube against the aorta with one hand while pulling the silk suture with the other hand. Aortic constriction was quickly released to avoid neurohumoral reflex changes in cardiac function (3, 4). Peak systolic pressure of isovolumetric heartbeats, which can be obtained with aortic occlusions, is a sensitive index of left ventricular contractility. A 3-F high-fidelity micromanometer (SPR-524, Millar Instruments, Houston, TX, U.S.A.) was inserted through an apical puncture wound into the left ventricular (LV) cavity, positioned at the midventricular level, and secured in place with a purse-string suture to measure LV pressure. The manometer was calibrated against a mercury column and zeroed after stabilization for 30 min in a water bath at body temperature. A limb electrocardiogram (DII) was recorded throughout.

After complete instrumentation, we allowed the animal preparation to stabilize for 30 min before the beginning of the experimental protocol. Recordings were made with respiration suspended at end expiration.

Parameters were converted on-line to digital data with a sampling frequency of 500 Hz. LV pressures were measured at end diastole and peak systole. Peak rates of LV pressure rise (dP/dt_{max}) and pressure fall (dP/dt_{min}) were measured as well. The relaxation rate was estimated with the time constant τ by fitting the isovolumetric pressure fall to a monoexponential function.

Anaesthetics ketamine hydrochloride (Imalgene 1000®), medetomidine hydrochloride (Domitor®) and xylazine hydrochloride (Rompum®) were obtained from Merial Portuguesa – Saúde Animal, Pfizer Saúde Animal, and Bayer, Portugal, respectively.

Papillary muscle studies

1. Experimental preparation

The study was performed in isolated right papillary muscles from the Control (n=41) and DOX-HF (n=15) groups one 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 mmol/L: NaCl 98; KCl 4.7; $MgSO_4.7H_2O$ 2.4; KH_2PO_4 1.2; glucose 4.5; $CaCl_2.2H_2O$ 1.8; $NaHCO_3$ 17; $C_3H_3NaO_3$ 15; CH_3COONa 5; atenolol 0.02) at 35 ºC 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 7.38-7.42.

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 plexi glass organ bath containing the above-described KR solution and 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 hours, muscles were stabilized. Bathing solutions were then replaced by corresponding KR solutions without calf serum and maximum physiologic length (L_{max}) was calculated. Protocols were initiated after obtaining two similar isotonic and isometric control twitches separated by a 10 min interval.

2. Experimental protocols

In a set of papillary muscles from Control (n=8) and DOX-HF (n=7) groups, isometric contractility-frequency relationships were obtained by plotting maximum velocity of tension rise against frequency of contraction. In summary, after an initial period of contraction at 0.6Hz, the frequency of stimulation was stepped up at 3-minute intervals to 1Hz, 2Hz, 3Hz and 4Hz.

Myocardial effects of increasing concentrations of ET-1 (0.1, 1, and 10 nM) were studied in rabbit papillary muscles from the: (i) Control Group with intact endocardial endothelium (EE) (n=9); (ii) Control Group with damaged EE (n=9); (iii) Control Group in the presence N^G-Nitro-L-Arginine (L-NNA; nitric oxide synthase inhibitor, 1µM, n=8); (iv) Control Group in the presence of Indomethacin (INDO; cyclooxigenase inhibitor, 1µM, n=7) and (v) DOX-HF Group (n=8).

The concentrations of ET-1 were selected on the basis of several studies showing that its physiological effects on contraction and distensibility of myocardial tissue preparations or whole heart preparations are exerted by concentrations in the nanomolar range (Shah *et al*. 1989, Firth *et al*. 1990, Leite-Moreira *et al*. 2003, Bras-Silva and Leite-Moreira 2006).

EE was damaged by briefly (1 sec) exposing the isolated papillary muscle to a weak solution (0.5%) of the detergent Triton X-100 (Brutsaert *et al.* 1988, Leite-Moreira and Bras-Silva 2004).

Chemicals were obtained from Sigma Chemical Company, St. Louis, Missouri.

3. Data analysis

Isotonic and isometric twitches were recorded and analyzed. Selected parameters include: resting tension (RT) at the beginning (RT_{beg}, mN/mm^2) and at the end (RT_{end}, mN/mm^2) of the twitch; 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).

When a pharmacological inhibitor (L-NNA or INDO) was used, the term baseline refers to the condition in the presence of those inhibitors before the addition of ET-1.

In the various protocols, results are given as percent change 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. Baseline performance of papillary muscles from Control and DOX-treated rabbits were compared with an unpaired t-test. Effects of increasing concentrations of ET-1 and of increasing stimulation frequencies on papillary muscles from Control and DOX-treated rabbits were analyzed with a repeated measures two-way ANOVA. Echocardiographic data of DOX-treated animals at the beginning and at the end of the study were compared with a paired t test. Hemodynamic measurements at baseline and after treatment with DOX or saline were analyzed with a repeated-measures two-way ANOVA. When significant differences were detected, the Student-Newman-Keuls was selected to perform multiple comparisons. Differences were considered to be significant when $P<0.05$.

RESULTS

1. Cardiac Hemodynamics and Echocardiography

The hemodynamic features of the experimental groups are summarized on Table 1. The DOX-HF group presented, in comparison with the Control group, a lower systolic pressure, dP/dt_{max} and peak systolic isovolumetric pressure. The left ventricular filling pressure, as estimated by left ventricular end-diastolic pressure, was increased in DOX-HF whereas the dP/dt_{min} was decreased and the relaxation time constant τ was increased in the DOX-HF (Table 1).

Additionally, in the DOX-HF group, the echocardiographic evaluation demonstrated a progressive increase of the end-diastolic (from 14.2±0.3 mm to 15.2±0.3 mm) and end-systolic (from 9.9±0.2 mm to 11.1±0.3 mm) short-axis diameters and a reduction in fractional shortening (from 32%±1% to 26%±1%) and ejection fraction (from 64%±1% to 56%±2%) of the left ventricle, consistent with the presence of dilated cardiomyopathy and HF. None of the other parameters measured significantly changed after doxorubicin treatment, namely heart rate (158 bpm ±6 *vs* 144 ±7 bpm), left wall thickness in diastole (2.26± 0.07mm *vs* 2.20± 0.05mm) and left wall thickness in systole (3.40±0.13mm *vs* 3.42± 0.11mm).

2. Papillary muscle studies

Mean values of the baseline contractile parameters in papillary muscles from the Control group with intact EE (n=32) and from the DOX-HF group (n=15) are shown in Table 2. Removal of the EE (n=9) resulted in a negative inotropic effect. Although baseline performance of rabbit papillary muscles was similar in the Control group with intact EE and in the DOX-HF group, contractility of papillary muscles from the Control group did not significantly decline with increasing stimulation frequency, between 1 Hz and 4 Hz, while the papillary muscles from the DOX-HF rabbits showed a significantly decrease in contractility over the same range of stimulation frequencies, indicative of contractile dysfunction and a reduced contractile reserve

(Figure 1). In the presence of an intact EE, ET-1 promoted concentration-dependent positive inotropic and lusitropic effects: AT increased 15.3±5.4%, 47.2±9.8% and 88.6±18.3%; dT/dt_{max}, 15.4±5.9%, 47.1±12.3% and 103.7±21.5% and dT/dt_{min}, 13.3±4.9%, 42.4±6.8% and 85.6±16.9% (at 0.1, 1, and 10 nM, respectively). These effects were maintained after damaging the EE, in the presence of L-NNA or INDO and in the DOX-HF Group (Figure 2).

Concerning the effects of ET-1 on myocardial distensibility, we found that RT significantly decreased after an isometric twitch in the presence of ET-1. Such a decrease was not significant at baseline and became progressively larger with increasing doses of ET-1 in muscles with intact EE. In fact, compared with its value at the beginning of the twitch (RT_{beg}), RT at the end of an isometric twitch (RT_{end}) decreased 3.2±1.3%; 6.0±1.6% and 8.8±2.7% in the presence of 0.1, 1, and 10 nM of ET-1, respectively (Figure 3). Such a decrease in RT reflects an increase in myocardial distensibility, because restoring the value of RT to its initial value results in an increase in the resting length of the muscle. No significant differences between RT_{end} and RT_{beg} were however found when ET-1 was given after damaging the EE or in papillary muscles from the DOX-HF group. Similarly, ET-1 did not significantly alter myocardial distensibility after blocking NO or prostaglandins release by L-NNA or INDO, respectively.

DISCUSSION

The present study showed that the increase in myocardial distensibility induced by ET-1 is absent in DOX-HF and is dependent on NO and prostaglandins release.

The progression of cardiac dysfunction was monitored echocardiographically to estimate morphologic and functional alterations during the development of HF. Hemodynamic studies performed one week after the last administration of DOX, also showed the presence of systolic and diastolic dysfunction in DOX-HF animals. 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 (Endoh 2004), contractility-frequency relationships were performed. We found that although baseline 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.

Positive inotropic and lusitropic effects of ET-1 have been previously described by several authors in various experimental preparations, although the magnitude of the effects varied among distinct animal species (Endoh 1998). Rabbits are one of the most sensitive animals to ET-1, which was one of the reasons for carrying out the experiments in this species. The magnitude of positive inotropic and lusitropic effects obtained in the present study is consonant with previously published data in rabbit papillary muscles (Li *et al.* 1991, Leite-Moreira and Bras-Silva 2004, Leite-Moreira *et al.* 2003, Bras-Silva and Leite-Moreira 2006). These inotropic and lusitropic effects of ET-1 were maintained after damaging EE, blocking NO and prostaglandins release and in the DOX-HF group. Previous studies *in vivo* and *in vitro* showed that the contractile effects of ET-1 were increased (Li and Rouleau 1996), attenuated (Mollmann *et al.* 2006), maintained (Bras-Silva and Leite-Moreira 2006) or even reversed (Kelso *et al.* 1996, Thomas *et al.* 1996, MacCarthy *et al.* 2000) in the presence of HF. This difference could be explained by the different methodological approaches, different animal species and different experimental models of HF.

In the present study, we therefore observed that despite the occurrence of baseline contractile dysfunction in failing hearts, baseline performance of papillary muscles was similar in control and doxorrubicin-treated animals. Furthermore, these muscles exhibited the same inotropic and lusitropic response to ET-1, but a distinct inotropic response to increasing stimulation rates, closer to the physiologic range. The negative force-frequency relationship is a well-known feature

of the failing myocardium that can be at least partially attributed to disturbed calcium homeostasis and energy imbalance (Endoh 2004). On the other hand, the contractile response to ET-1 involves distinct cellular mechanisms, which might explain its similar effects in the normal and failing myocardium. Furthermore, ET-1 has the ability to increase cardiac contractile efficiency by lowering ATPase activity (McClellan *et al*. 1996) and oxygen consumption (Takeuchi *et al*. 2001) and was considered essential for the contractile efficiency of the failing myocardium (Sakai *et al*. 1996).

With regard to the effects of ET-1 on the diastolic properties of the myocardium, we found that the decrease in resting tension (increase in myocardial distensibility) observed after an afterloaded twitch in presence of ET-1 was not present in the failing myocardium. We also confirmed that damaging the EE also blocked this effect confirming previous observations (Bras-Silva and Leite-Moreira 2006). In previous studies we have also shown that this effect of ET-1 on myocardial distensibility was mediated by ET_A receptor stimulation (Leite-Moreira *et al.* 2003), and dependent of endothelial ET_{B1} receptor's activity, even if the direct stimulation of either endothelial ET_{B1} or myocardial ET_{B2} receptors did not elicit any effect on this parameter (Bras-Silva and Leite-Moreira 2006). If we take into account that endocardial endothelium is dysfunctional in the DOX-HF model (Bras-Silva *et al.* 2006) and that the acute effects of ET-1 on myocardial distensibility are blocked when the EE is damaged, it seems plausible that the blunted effects of ET-1 on myocardial distensibility in the failing myocardium would be explained by EE dysfunction.

Once NO and prostaglandins are two of the most important endothelial mediators and they are known to be released by the endothelium in response to ET_{B1} receptor stimulation (de Nucci *et al.* 1988, Thiemermann *et al.* 1989, Filep *et al.* 1991, Hirata *et al.* 1993 Leite-Moreira and Bras-Silva 2004), which also influences ET-1 effect on myocardial distensibility (Bras-Silva and Leite-

Moreira 2006), we investigated how these two agents modulate ET-1 effects. We found that similarly to what happened after damaging EE, after blocking NO or prostaglandins release the ET-1 induced decrease in resting tension (increase in distensibility) was not observed.

NO has been shown to increase diastolic distensibility (Paulus *et al.* 1994, Paulus and Shah 1999), effect that seems to be mediated by reduction of myofilamentary calcium sensitivity because of phosphorilation of troponin I by cGMP- dependent protein kinase (Shah and MacCarthy 2000). Direct myocardial actions of prostaglandins are still not clear. With regard inotropy both negative (Schor and Hohlfeld 1992) and positive (Mohan *et al.* 1995) effects were shown in isolated papillary muscles. Regarding lusitropy prostaglandins were recently shown to preserve early active diastolic relaxation (Kisch-Wedel *et al.* 2005) and to blunt the premature onset of tension decline promoted by ghrelin (Soares *et al.* 2006). This two agents, NO and prostaglandins, have also been implicated in the negative inotropic effects resulting from selective ET_{B1} receptor stimulation (Leite-Moreira and Bras-Silva 2004). So it seems that independently of the direct actions of each of these endothelial agents, they are able to regulate both systolic and diastolic effects of ET-1.

Concerning the pathophysiologic relevance of our findings, we must point out that a lower resting tension of the cardiac muscle indicates the ventricle can reach higher filling volumes at lower filling pressures, which is undoubtedly a quite powerful adaptation mechanism. These acute beneficial ET-1 effects on diastolic function seem to be overcome by its role in progression to cardiac fibrosis and ventricular remodelling when its levels remain chronically elevated (Brunner *et al.* 2006). Additionally, the results of the present study emphasize that humoral influences on diastolic cardiac function are modulated by the interaction with endocardial endothelial mediators, such as NO and prostaglandins, which being altered in the failing heart might provide new elements for the comprehension of the pathophysiology of HF. Finally, 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 (Monnet and Chachques 2005). In this context, our findings might also be relevant to better understand the pathophysiology of DOX-induced cardiomyopathy so that efficient protective and/or therapeutic strategies can be developed in patients treated with this chemotherapeutic agent.

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LEGENDS

Figure 1. Contractile response of rabbit papillary muscles from the Control group (n=8) 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 1Hz and 4 Hz, whereas in the DOX-HF group, muscles responded in the opposite way. $P < 0.05$: *, *vs.* baseline; #, *vs.* control.

Figure 2. Concentration-response curves for the effect of ET-1 on the contractile parameters of rabbit papillary muscles in various experimental conditions: Control group with intact endocardial endothelium (EE; full circles, n=9); Control group with damaged EE (open circles, n=9); Control group with intact EE and in the presence of N^G-Nitro-L-Arginine (L-NNA, open triangles, n=8) ; Control group with intact EE and in the presence of Indomethacin (INDO, full squares, n=7) and Doxorrubicin-induced heart failure group (DOX-HF; full triangles, n=8). AT, active tension; dT/dt_{max}, maximum velocity of tension rise; dT/dt_{min}, maximum velocity of tension decline. Mean ±SE; percentage of baseline. * $P< 0.05$ *vs.* baseline.

Figure 3. Concentration-response curves for the effect of ET-1 on resting tension (RT) of rabbit papillary muscles in various experimental conditions: Control group with intact endocardial endothelium (EE; full circles, n=9); Control group with damaged EE (open circles, n=9); Control group with intact EE and in the presence of N^G-Nitro-L-Arginine (L-NNA, open triangles, n=8) Control group with intact EE and in the presence of Indomethacin (INDO, full squares, n=7) and Doxorrubicin-induced heart failure group (DOX-HF; full triangles, n=8). Mean ±SE; percentage of baseline. * $P< 0.05$ *vs.* baseline.

Figure 1.

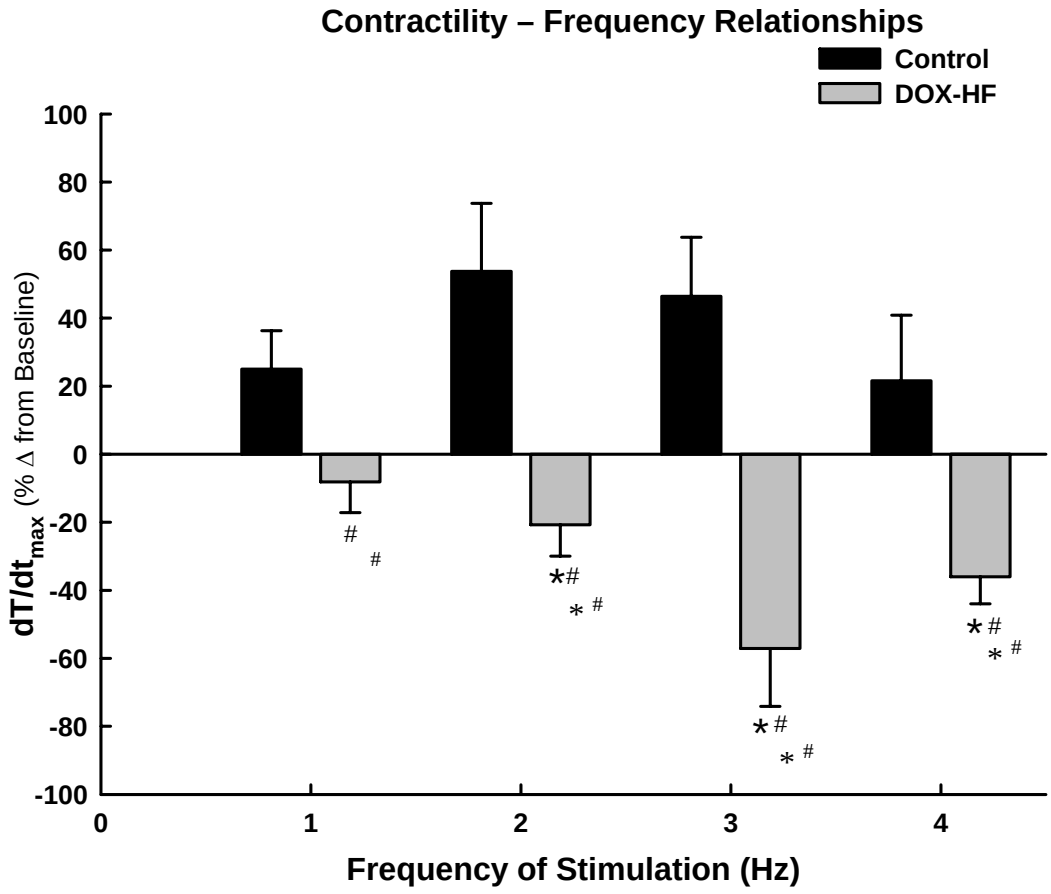
Contractility – Frequency Relationships
Control
DOX-HF
100
80
60
40
20
0
-20
-40
-60
-80
-100
dT/dtmax (% Δ from Baseline)
0
1
2
3
4
Frequency of Stimulation (Hz)

Figure 2.

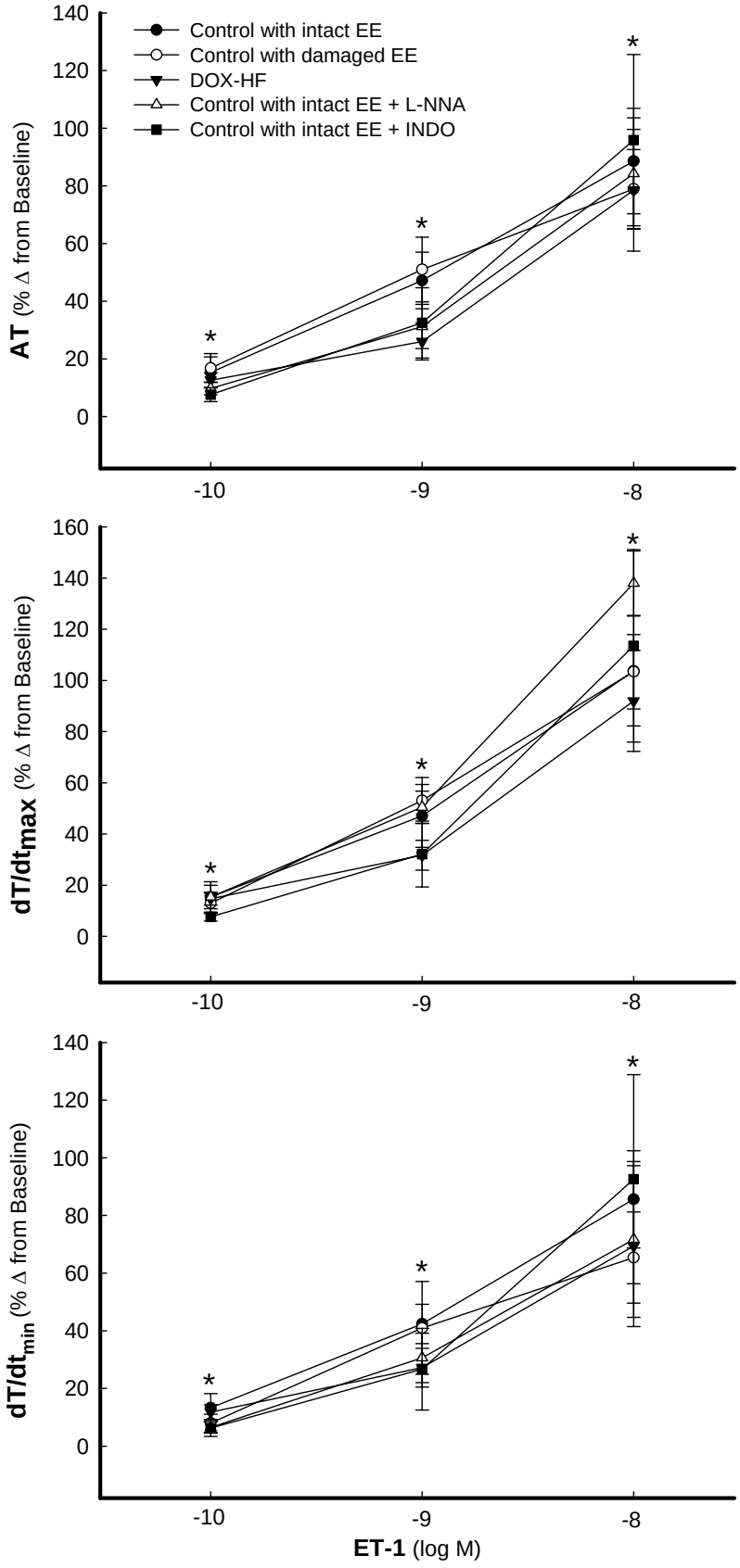
Control with intact EE
Control with damaged EE
DOX-HF
Control with intact EE + L-NNA
Control with intact EE + INDO
AT (% Δ from Baseline)
dT/dtmax (% Δ from Baseline)
dT/dtmin (% Δ from Baseline)
ET-1 (log M)
-10
-9
-8
*

Figure 3.

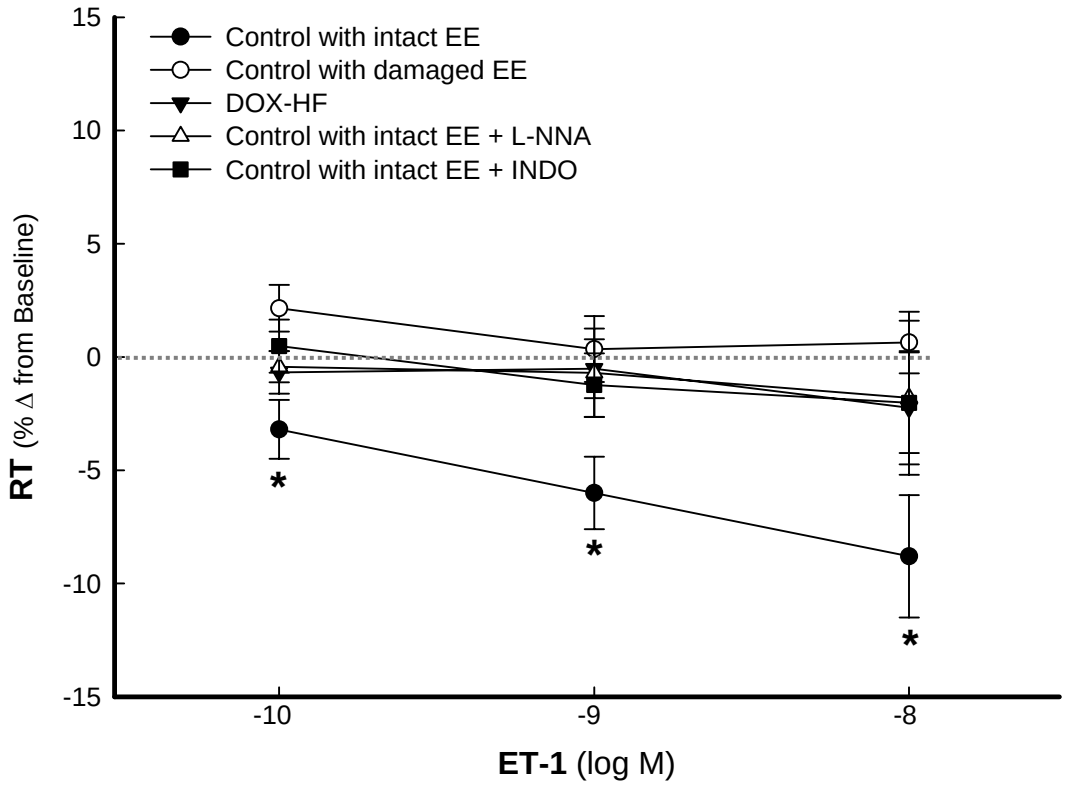
Control with intact EE
Control with damaged EE
DOX-HF
Control with intact EE + L-NNA
Control with intact EE + INDO
15
10
5
0
-5
-10
-15
RT (% Δ from Baseline)
-10
-9
-8
ET-1 (log M)
*
*
*

Table 1. *Hemodynamic data of rabbits from the Control and Doxorubicin-Induced Heart Failure (DOX-HF) Groups*

	Control Group (n=6)	**DOX-HF Group (n=9)**
LVSP, mmHg	64.8±4.7	47.7±9.7*
LVEDP, mmHg	1.2±0.3	2.28±0.34*
dP/dt_{max}, mmHg/s	3026.0±244.0	1274±266.0*
dP/dt_{min}, mmHg/s	-2004.0±378.0	-992.0±171.0*
LVP_{ISO}, mmHg	148.9±9.2	84.6±13.5*
τ, ms	36.6±7.7	68.9±7.1*

Values are mean ± SE. LVEDP and LVSP, left ventricular end-diastolic and systolic pressures, respectively; dP/dt_{max} and dP/dt_{min}, peak rates of ventricular pressure rise and fall, respectively; LVP_{ISO}, peak systolic isovolumetric pressure; τ, time constant of isovolumetric relaxation. *P<0.05 *vs.* Control group.

Table 2. *Mean values of the baseline contractile parameters in papillary muscle from the Control and Doxorubicin-Induced Heart Failure (DOX-HF) Groups*

Contractile Parameter	Control Group		DOX-HF Group
	With EE (n=32)	Without EE (n=9)	(n=15)
AT (mN/mm^2)	23.3 ±2.7	17.4 ±1.9*	26.3±4.3
dT/dt_{max} ($mN/mm^2/sec$)	163.5±17.1	112.5±11.6*	164.5±21.3
dT/dt_{min} ($mN/mm^2/sec$)	-133.1±15.3	-95.2±9.6*	-137.8±22.2
PS (% of L_{max})	12.0±0.1	9.0±0.1*	11.0±0.1
dL/dt_{max} (L_{max}/sec)	0.89±0.1	0.61±0.06*	0.71±0.05
dL/dt_{min} (L_{max}/sec)	-3.20±0.40	-2.01±0.2*	-2.43±0.2

Values are means ± SE. 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. * $P < 0.05$ *vs.* Control Group with intact EE.