Endothelin-1 inhibits the neuronal norepinephrine transporter in hearts of male rats

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Abstract

Objective: Endothelin-1 (ET-1) potentiates norepinephrine (NE)-induced contractile responses. An impairment of cardiac NE re-uptake by the neuronal NE transporter (NET) contributes to an increased NE net release in failing hearts. We hypothesized that both phenomena are caused by ET-1-mediated inhibition of NET.

Methods: [3H]-NE-uptake, electrical field stimulation-evoked NE overflow and left ventricular contractility (LV-dp/dtmax) were measured in isolated perfused rat hearts. NET density on cardiac plasma membranes was determined by [3H]-mazindol binding. Experimental heart failure in rats was induced by transverse aortic constriction (TAC).

Results: ET-1 inhibited cardiac [3H]-NE-uptake in a concentration- and time-dependent manner. The endothelin A receptor (ETA) antagonist BQ123 but not the endothelin B receptor (ETB) antagonist BQ788 abolished ET-1-induced reduction of [3H]-NE-uptake. Likewise, ET-1, but not the ETB agonist sarafotoxin S6c, enhanced the stimulated overflow of endogenous NE. In contrast, ET-1 inhibited the stimulated NE overflow during NET blockade (exocytotic NE release) via activation of ETB. In isovolumically contracting healthy hearts, ET-1 potentiated the NE- but not isoprenaline-induced increase in LV-dp/dtmax. Since isoprenaline is not a NET substrate, the enhanced LV-dp/dtmax response to NE thus depends on NET. In TAC rats, ETA antagonism by darusentan improved both impairment of cardiac [3H]-NE-uptake and reduction of [3H]-mazindol binding sites.

Conclusion: ET-1 inhibits cardiac NE re-uptake via ETA but attenuates exocytotic NE release via ETB, resulting in opposite effects on cardiac NE net release. In healthy hearts, ETA-mediated inhibition of NE re-uptake exceeds ETB-mediated silencing of NE release and potentiates the NE-induced increase in left ventricular contractility. In TAC rats, endogenous ET-1 impairs NE re-uptake and promotes sympathetic overstimulation of failing hearts.

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1. Introduction

Endothelin-1 (ET-1) is a ubiquitous 21 amino-acid peptide discovered as a potent vasoconstrictor [1]. Among various functions on the cardiovascular, pulmonary, renal and neuroendocrine systems [2], ET-1 has also the ability to potentiate norepinephrine (NE)-induced effects on vascular smooth muscle cells [3–8]. In congestive heart failure (CHF), elevated ET-1 plasma levels have been shown to promote sympato-adrenergic overstimulation of failing hearts [9–12], but the underlying mechanisms are not fully
understood. Though potentiation of NE-induced effects by ET-1 might be explained by increased calcium sensitivity of the contractile apparatus (postsynaptic effect), there is also evidence for a presynaptic mechanism of ET-1 on NE release from the sympathetic nerve ending [13–20].

The number of NE molecules that bind to postsynaptic adrenoceptors is not only determined by the release of the amine from the peripheral nerve ending but also by its elimination. After its release into the synaptic cleft more than 90% of NE is removed by neuronal re-uptake (uptake1) via the NE transporter (NET), whereas extraneuronal uptake (uptake2) of the amine plays only a minor role in the heart [21]. Any impairment of NET-mediated NE re-uptake would markedly increase the “effective” NE concentration at postsynaptic adrenoceptors. An impairment of cardiac NE re-uptake by NET downregulation contributes to the increased cardiac net release of NE in CHF, which is associated with depletion of cardiac NE stores, down-regulation of cardiac beta-adrenoceptors and profound alterations of post-receptor signal coupling [21–26]. Clinical studies have shown that impaired cardiac NE re-uptake is associated with poor prognosis (reflected by worsening of CHF and increasing incidence of sudden death) [27]. In an experimental CHF model, we have recently reported that an impairment of NE re-uptake is mediated by posttranscriptional NET downregulation [26]. In cell culture, it has been shown that activation of protein kinase C (PKC) induces translocation of NET from the plasma membrane to the cytoplasm [28,29].

We hypothesized that activation of presynaptic receptors, which are associated with PKC activation, might be involved in NET downregulation. Here we demonstrate that ET-1 via activation of endothelin-A-receptors (ET_{A}) potentiates the NE-induced increase in cardiac contractility by inhibition of NET. Moreover, we report that ET_{A} antagonism improves NET function in experimental CHF.

2. Materials and methods

The investigation conforms with 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) and was approved by the authorities of the Regierungspräsidium Karlsruhe, Germany.

2.1. Isolated heart perfusion

Wistar rats were anesthetized with thiopental (100 mg/kg i.p.). The hearts were rapidly cut out, rinsed in ice-cold buffer and the aorta was cannulated for perfusion according to Langendorff (1895). Within one experiment, 8–12 spontaneously beating hearts were perfused simultaneously at a constant coronary flow and a constant temperature of 37.5 °C. The perfusion medium was a modified Krebs–Henseleit solution (composition in mmol/L: NaCl 125, NaHCO_{3} 16.9, Na_{2}HPO_{4} 0.2, KCl 4.0, CaCl_{2} 1.85, MgCl_{2} 1.0, glucose 11, EDTA 0.027). The buffer was gassed with 95% O_{2} and 5% CO_{2} and the pH was adjusted to 7.4.

Cardiac [^{3}H]-NE uptake was determined as described previously [26]. Briefly, a bolus of [^{3}H]-NE (1 mL, 3 μCi, 100 pmol NE; Amersham-Buchler, Braunschweig, Germany) was injected into the perfusion system and proportionally distributed to the hearts and blank channels. Radioactivity was measured in the effluent. The amount of [^{3}H]-NE, which was extracted by the hearts (uptake), is expressed as the percentage of radioactivity measured in the blank channels.

Overflow of endogenous NE was evoked by electrical field stimulation as described previously [26]. Briefly, isolated perfused hearts were stimulated with bipolar platinum electrodes (1 min, 4 Hz, 5 V). Two or three subsequent electrical stimulations were applied with an interval of 10 min in the presence of atropine (1 μmol/L) to prevent vagal activation. To distinguish between exocytotic and net release of NE, experiments were performed in the absence and presence of the NET inhibitor desipramine (DMI). Drugs were added 10 min prior to stimulations as indicated. Endogenous NE in the coronary venous effluent were determined by HPLC and electrochemical detection.

Coronary perfusion pressure was measured via a Statham P23 b transducer (Gould, Cleveland, OH, USA) connected to a side arm of the perfusion system.

Isovolumically contracting hearts were prepared according to Hampton et al. [30]. Briefly, isolated hearts were perfused at a constant coronary flow. A left atrial incision was made to expose the mitral annulus, through which a water-filled latex balloon attached to a catheter system was passed into the left ventricle. The distal end of the catheter was connected via a larger catheter to another Statham transducer to record the left ventricular (LV) pressure (LVP). LV-dp/d_{t_{\text{max}}} and mean perfusion pressure (PP) were calculated to assess cardiac function and coronary vasoconstriction.

The following agents were used: ET-1, BQ123, BQ788, sarafotoxin S6c, S-nitrosoacaptopril (Calbiochem, Schwalbach, Germany), methoxamine, nicotine, bradykinin, angiotensin II, atropine, DMI, NE and isoprenaline (Sigma, Munich, Germany).

2.2. Experimental heart failure

Transverse aortic constriction (TAC) was performed as described previously [26]. Briefly, Wistar rats (90 g) were anesthetized with ketamine and diazepam. After thoracotomy the ascending aorta was partially occluded using tantalum hemostatic clips with a defined internal diameter of 0.71 mm. Some animals were treated with darusentan (30 mg/kg/day in the drinking water; kindly provided from Knoll AG, Ludwigshafen, Germany). A control group was sham operated. After 28 days the rats were anesthetized with thiopental. Blood samples were taken from the femoral vein for determination of plasma proANP. Lungs were excised.
for measuring the lung wet weight. Hearts were excised, weighed and used for (i) isolated heart perfusion, (ii) determination of LV-ET-1 or (iii) plasma membrane preparations and subsequently [3H]-mazindol binding.

2.3. Plasma proANP and tissue ET-1

Plasma proANP and tissue ET-1 were determined using ELISA according to the manufacturers instructions (Biomedica, Vienna, Austria).

2.4. [3H]-mazindol binding

Plasma membranes of left ventricles were prepared as described before [26]. Radioligand binding assays were performed in a total volume of 250 μL containing 50 μg of plasma membranes and increasing concentrations of [3H]-mazindol (specific activity 52, 5 Ci/mmoll; NEN, Dreieich, Germany) as a specific ligand. Non-specific binding was determined by measuring the residual binding in the presence of DMI (100 μmol/L). The incubation was carried out at 30 °C and terminated by rapid vacuum filtration through a MultiScreenHTS-FB filter plate (Millipore, Schwalbach, Germany). All experiments were performed in triplicate. The remaining filter radioactivity was determined, and Bmax was calculated using GraphPad Prism version 4.00 software (GraphPad Software, San Diego, California, USA).

2.5. Animals

For all experiments male Wistar rats were used. 180 healthy animals (180–250 g) were used for cardiac [3H]-NE uptake (n = 116), electrical field stimulation (n = 42) or preparation of isovolumically contracting hearts (n = 22). 52 male Wistar rats (90 g) underwent sham (n = 18) and TAC surgery (n = 34). 13 of TAC-operated rats were treated with the ETA antagonist darusentan. Sham- and TAC-rats were used for determination of LV-ET-1 (n = 8), cardiac [3H]-NE uptake (n = 25) and LV-[3H]-mazindol binding (n = 19).

2.6. Statistical methods

The results are expressed as means ± SEM. Statistical analysis was performed with the GraphPad Prism version 4.00 software. Intra-individual differences were tested with the paired t test and ANOVA for repeated measurements. Differences between groups were tested with the Mann–Whitney test. A p-value of <0.05 was considered significant.

3. Results

3.1. ET-1 inhibits NE re-uptake via ETA

Activation of presynaptic receptors by 30 min perfusion with 100 μmol/L nicotine (27.2 ± 2.2 vs. 30.2 ± 1.7%; n = 5), 1 μmol/L bradykinin (27.4 ± 1.0 vs. 30.6 ± 1.4%; n = 5), 1 μmol/L methoxamine (28.2 ± 1.6 vs. 26.2 ± 2.0%; n = 4) or 100 nmol/L angiotensin II (27.2 ± 1.8 vs. 28.8 ± 3.6%; n = 5), all known to augment NE net release, did not reduce cardiac [3H]-NE-uptake in isolated perfused rat hearts as compared to a control perfusion. In contrast, 30 min perfusion with 1 nmol/L ET-1 reduced cardiac [3H]-NE-uptake markedly by more than 30% (21.2 ± 0.8 vs. 31.0 ± 0.9%; n = 5; p < 0.05). There was no difference between ET-1-and tyrode-perfused hearts in the residual uptake of [3H]-NE after specific blockade of NET with 1 μmol/l DMI, confirming that the ET-1-mediated effect was entirely due to a reduced uptake via NET (data not shown). ET-1 inhibited cardiac uptake of [3H]-NE in a concentration-and time-dependent manner (Fig. 1A): Significant reductions of [3H]-NE-uptake were obtained by 10 nmol/L ET-1 after 5 min, by 1 nmol/L after 15 min and by 100 pmol/L after 30 min. [3H]-NE-uptake was inhibited by maximal 85% in response to 10 nmol/L (after 30 min), by 50% to 1 nmol/L (after 45 min) and by

![Fig. 1. Cardiac [3H]-NE uptake. (A) Effects of indicated ET-1 concentrations and perfusion periods on cardiac [3H]-NE uptake. n ≥ 5 in each group; *p < 0.05 vs. both time-matched control perfusion (i.e. separate group) and respective control before perfusion with ET-1. (B) Effects of ET-1 (1 nmol/L) in the absence (n = 12) and presence of 100 nmol/L BQ123 (n = 9) or BQ788 (n = 10) after a perfusion period of 15 min. Also shown is the effect of 45 min perfusion with the ETA agonist sarafotoxin 6c (StxS6c; 10 nmol/L; n = 5).](https://academic.oup.com/cardiovascres/article-abstract/67/2/283/284635)
25% to 100 pmol/L (after 45 min) ET-1. Perfusion with a concentration of 30 pmol/L ET-1 did not cause a significant decrease in cardiac $[^{3}H]$-NE-uptake.

Pretreatment with the specific ETA antagonist BQ123, but not with the ETB antagonist BQ788, abolished the ET-1-induced reduction of cardiac $[^{3}H]$-NE-uptake (Fig. 1B), identifying ETA as the predominant endothelin receptor subtype in regulation of NE re-uptake. Likewise, sarafotoxin S6c (StxS6c), a specific ETB agonist, did not reduce cardiac $[^{3}H]$-NE-uptake after perfusion periods of 15, 30 (data not shown) and 45 min (Fig. 1B). Perfusion of BQ123 or BQ788 alone did not affect cardiac $[^{3}H]$-NE-uptake (data not shown).

One could argue that the ET-1-induced phenomenon is nonspecifically mediated by ETA-mediated severe vasoconstriction and subsequent ischemia. Therefore, cardiac $[^{3}H]$-NE-uptake experiments were performed in the presence of the vasodilator S-nitrosothiopropril (S-NC). S-NC completely normalized ET-1-induced increase in perfusion pressure (Fig. 2A) but did not prevent ET-1-induced inhibition of cardiac $[^{3}H]$-NE-uptake (Fig. 2B). S-NC by itself did not affect cardiac $[^{3}H]$-NE-uptake (Fig. 2B), also indicating that the coronary vascular tone does not affect $[^{3}H]$-NE-uptake.

3.2. Effects of ET-1 on stimulated overflow of endogenous NE

Given that ET-1 inhibits cardiac NE re-uptake, an enhanced NE overflow after stimulation of cardiac sympathetic nerves would be expected. In fact, after 10 min preperfusion with 10 nmol/L ET-1, but not with StxS6c, electrical field stimulation-evoked overflow of endogenous NE was increased as compared to preperfusion with tyrode (Fig. 3). To rule out that the enhanced NE overflow was due to an increase in exocytotic NE release, the same experiment was performed in the presence of the NET inhibitor DMI. Under these conditions ET-1 did not lead to an enhanced NE overflow. In contrast, presence of DMI exerted the opposite effect: NE overflow was markedly inhibited by ET-1, suggesting an additional inhibitory effect of ET-1 on exocytotic NE release (Fig. 3). In contrast to the inhibitory effect of ET-1 on cardiac $[^{3}H]$-NE-uptake, the latter effect was not blocked by BQ123 but significantly attenuated by BQ788 and mimicked by StxS6c, indicating that the inhibitory effect on NE overflow is mediated by ETB (Fig. 3).

3.3. ET-1 potentiates NE-induced increase in LV-dp/dtmax via NET

To further investigate the functional relevance of ET-1-induced reduction of cardiac NE re-uptake, the increase in LV-dp/dtmax in response to NE and the beta-adrenergic agonist isoprenaline (Iso) were measured in the absence and the presence of ET-1. Since Iso is not taken up by NET [24], its removal from the circulation occurs in a way that is

Fig. 2. Perfusion pressure and cardiac $[^{3}H]$-NE uptake. (A) Representative recordings of perfusion pressure during perfusion with tyrode and subsequent ET-1 (1 nmol/L) in the absence and presence of S-nitrosothiopropril (10 µmol/L; S-NC), (B) Cardiac $[^{3}H]$-NE uptake before (open bars) and 30 min after perfusion with ET-1 (1 nmol/L) in the absence ($n=5$) and presence of S-NC (10 µmol/L; $n=9$) and after S-NC (10 µmol/L; $n=4$) alone (black bars).

Fig. 3. Electrical field stimulation of isolated perfused rat hearts. The first 4 bars show stimulated NE overflow before (open bars) and 10 min after perfusion with 10 nmol/L ET-1 ($n=8$) and StxS6c ($n=7$) (black bars). The next 8 bars show NE overflow in the presence of the specific NET inhibitor DMI before (open bars) and after 10 min perfusion with 10 nmol/L ET-1 without ($n=6$) and with preperfusion of BQ123 ($n=7$) or BQ788 ($n=6$). Also shown is the NE overflow in the presence of DMI before (open bar) and 10 min after perfusion with 10 nmol/L StxS6c ($n=6$) (black bar).
similar to NE after NET blockade. ET-1 alone (100 pmol/L) increased LV-\(\text{dp/dt}_{\text{max}}\) after 30 min preperfusion by 25% (2.5 ± 0.1 vs. 1.7 ± 0.1 mmHg/ms, \(n = 13, p < 0.05\)). The additional increase in LV-\(\text{dp/dt}_{\text{max}}\) in response to augmenting concentrations of NE exceeded the increase in LV-\(\text{dp/dt}_{\text{max}}\) that was exerted by NE (0.1 and 1 μmol/L) in the absence of ET-1 (Fig. 4A). In contrast, preperfusion of ET-1 did not potentiate the Iso-induced increase in LV-\(\text{dp/dt}_{\text{max}}\) at any Iso concentration (Fig. 4B).

### 3.4. \(\text{ET}_{\text{A}}\) antagonism attenuates NET downregulation in experimental heart failure

To investigate whether an activated endogenous endothelin system downregulates NET in experimental CHF, TAC rats were treated with the specific \(\text{ET}_{\text{A}}\) antagonist darusentan. In accordance with our previous findings [26, 4 weeks after surgery TAC rats developed increased heart weight/body weight and lung wet weight/body weight ratios as well as an impaired cardiac \([\text{H}]\)-NE-uptake and reduced NET binding sites (Table 1). As a neurohormonal marker for the severity of CHF, plasma proANP was more than tripled in TAC rats as compared to sham rats (Table 1). Elevated LV-ET-1 levels confirmed an activation of the local cardiac endothelin system (Table 1). Darusentan did not lower the organ weights or the plasma proANP level in TAC rats. We observed rather a slight but not significant increase in heart and lung wet weight/body weight ratios and a significant 36% elevation of plasma proANP as compared to non-treated TAC rats (Table 1). This is most likely due to a darusentan-induced lowering of preload below a level that is required to overcome the hemodynamic burden of aortic stenosis. In contrast, darusentan significantly attenuated both the impairment of \([\text{H}]\)-NE-uptake and the reduction of NET binding sites (Table 1).

### 4. Discussion

In this study we report that ET-1 inhibits NET function through \(\text{ET}_{\text{A}}\) activation. We demonstrate that potentiation of the NE-induced increase in LV contractility by ET-1 is NET-dependent. Moreover, evidence is provided that down-regulation of NET in failing hearts is mediated by an activated endogenous endothelin system.

#### 4.1. Potentiation of NE-induced increase in LV contractility—postsynaptic versus presynaptic

It has been shown that pretreatment of mesenteric [8,20,31], mammary [3] and pulmonary arteries [19] or the aorta [4] with threshold concentrations of ET-1 (0.1–0.3 nmol/L) potentiates NE-induced contractile responses. Early

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**Table 1**

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<th>Transverse aortic constriction (TAC) of male Wistar rats</th>
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<td>Plasma proANP (fmol/mL)</td>
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<td>LV-[\text{H}]-mazindol binding sites</td>
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Left ventricular (LV)-ET-1, heart weight/body weight ratio, lung wet weight/body weight ratio, plasma proANP, cardiac \([\text{H}]\)-NE uptake and LV-[\text{H}]-mazindol binding sites were determined 4 weeks after surgery. Starting with the day of surgery, 13 TAC rats received the specific ET\(_{\text{A}}\) antagonist darusentan (DARUS, 30 mg/kg/day in the drinking water). *p < 0.05 vs. sham; \#p < 0.05 vs. TAC.
studies reported that ET-1 attenuates NE release and contractile responses due to sympathetic nerve stimulation, whereas it enhances contractile responses due to NE [8,19,20]. These findings imply that ET-1 mediates inhibitory effects on presynaptic neurotransmission and activating effects on postsynaptic noradrenergic signaling. Many studies confirmed the finding that ET-1 inhibits NE release through activation of ETA [13–15,32–34]. However, the nature of the modulatory role of ET-1 on sympathetic neurotransmission seems to be more complex, since other studies have shown enhancing or neutral effects of ET-1 on NE release or nerve-stimulated pressure responses [14–19]. Most of the studies, that investigated effects of ET-1 on NE- or stimulation-induced responses, used preparations of isolated arteries or the adrenal gland. To our knowledge no data are available about presynaptic effects of ET-1 on cardiac sympathetic neurotransmission and its potentiality of NE-induced increase in cardiac contractility. Here we show in a Langendorff preparation that besides an ETB-mediated inhibitory effect on cardiac exocytotic NE release, ET-1 also reduces NE re-uptake via ETA, resulting in an enhanced NE net release within the heart. The observation, that ET-1 potentiates only the NE- but not the Iso- (that is not taken up by the NET [24]) induced increase in LV contractility, strongly suggests that this identified presynaptic role of ET-1 might enhance NE-induced effects on cardiac function.

4.2. Exocytotic NE release, NE re-uptake and NE net release—ETA versus ETB

Opposite results regarding NE release after pretreatment with ET-1 have been described [13–19]. We observed an increase in stimulated NE overflow after preperfusion with ET-1. But in the presence of the specific NET inhibitor DMI we found a marked reduction via ETB. This confirms that the increase in the absence of DMI is mediated by ET-1-induced reduction of NE re-uptake via ETA. But it also implies that in cardiac sympathetic neurotransmission the inhibitory effect of ET-1 on NE re-uptake exceeds the inhibition of NE release with the functional consequence that ET-1 increases NE net release. To our knowledge, in most of the studies, that evaluated effects of ET-1 on NE release, no NET inhibitor was added to the perfusion system. Therefore, those studies did not distinguish between exocytotic NE release and NE re-uptake. Consequently, dependent on the ETA/ETB balance within the investigated tissue, the measured NE net release after pretreatment with ET-1 was inhibited, enhanced or neutralized. In accordance with our results, one study, that investigated NE release in the presence of DMI, reported a marked inhibition of NE release via ETB [32]. Taken together, we conclude that the balance between ETB-mediated inhibition of exocytotic NE release and ETA-mediated inhibition of NE re-uptake regulates NE net release and subsequently activation of postsynaptic adrenergic signaling.

4.3. Role of endogenous ET-1 for NET downregulation in CHF

As shown previously, impairment of NE re-uptake in an experimental model of CHF is mediated by posttranscriptional downregulation of NET per nerve ending, but the underlying presynaptic mechanism remained unclear [26]. Interestingly, it has been shown that PKC activation in cultured cells leads to translocation of NET or similar neurotransmitter transporters (dopamine transporter) from the plasma membrane to the cytosol and subsequently to its degradation [29,35]. Preliminary data (not shown) suggest that ET-1 inhibits NE re-uptake also through a PKC-dependent pathway. Because the experiments shown in this study were done in a Langendorff preparation, the observed NET modulation is also caused by a posttranscriptional mechanism. As shown before [26], rats with TAC are known to develop an impaired NE re-uptake without a reduction of sympathetic nerve density [26]. In this study, we show that in the same CHF model ETA antagonism improved both NET function and binding sites. Since TAC rats did not show any improvement in other CHF markers, the observed increase in NET function and density is rather due to a direct effect on sympathetic nerve endings and not secondary due to an improved cardiac function. Recent studies provided evidence that endogenous ET-1 contributes to elevated NE plasma levels and sympathetic nerve activity in experimental CHF [11,12]. The present study suggests that NET downregulation and subsequent an increased NE net release in failing hearts is mediated by activation of presynaptic ETA on cardiac sympathetic nerve endings. We can rule out that ET-1 acts through modulation of the central sympathetic tone on NET function, because the used Langendorff model was independent of central control. There is still a possibility that ET-1 activates the ETA on cardiomyocytes or other cardiac cell types, which in turn induces secretion of unknown factors that mediate the described effects. Recently, it has been shown that cardiomyocyte-specific ETA-knockout mice exhibit normal heart growth and normal hypertrophic and contractile responses due to angiotensin II or Iso [36]. However, we cannot completely rule out that ETA of other cardiac cell types (e.g. fibroblasts or endothelial cells) affects NET on sympathetic cells indirectly. Nevertheless, we anticipate that the presynaptic ETA on cardiac sympathetic cells regulates NET function and consequently cardiac remodeling in CHF.

4.4. Clinical implications

Both elevated plasma levels of ET-1 and an impaired NE re-uptake are predictors of mortality in patients with CHF [37]. The results of the present study suggest that a selective ETA blockade attenuates sympathetic overstimulation of failing hearts via an enhanced NE re-uptake and a preserved ETA-mediated inhibition of exocytotic NE release. Likewise, it has been shown that the ETB agonist StxS6c protects
against ischemia-induced arrhythmias and decreases myocardial infarct size [38,39]. Although experimental studies reported beneficial effects of endothelin blockade on survival in CHF [40], long term treatment with the non-selective ETα and ETβ inhibitor bosentan failed to reduce morbidity or mortality in CHF patients [41]. Moreover, treatment of CHF patients for 6 months with the specific ETα antagonist darusentan did not improve cardiac remodeling (EARTH Trial) [42]. The neutral result of the EARTH Trial could be explained by the background treatment. Whereas ACE inhibitors and AT1 antagonists are known to decrease local cardiac ET-1 production [43], beta-blockers already attenuate sympathetic overstimulation of failing hearts. Nevertheless, ETα antagonists might be promising drugs in the management of CHF patients who are intolerant to ACE inhibitors and/or beta-blockers. Moreover, compared to beta-blockers, ETα antagonists would have the advantage that they potentially prevent an impairment of NE re-uptake and consequently depletions of cardiac NE stores in the sympathetic nerve ending [26], therefore ultimately preserving noradrenergic responses to stress and exercise [44].

5. Summary

This study demonstrates that ET-1 impairs cardiac NET function, resulting in a potentiated response to NE-induced LV contractility. Evidence is provided that downregulation of NET in failing hearts is at least in part mediated by an activated endogenous endothelin system.

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