Concentric left ventricular remodeling in endothelial nitric oxide synthase knockout mice by chronic pressure overload

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Abstract

Objective: Heart failure as a consequence of sustained hemodynamic overload is among the most prevalent diseases in developed countries. The aim of the present study was to investigate the specific role of endothelial nitric oxide synthase (eNOS) in pressure overload-induced left ventricular (LV) hypertrophy.

Methods and results: Chronic pressure-overload LV hypertrophy was induced by abdominal aortic banding (AC) in wild-type (WT) and eNOS−/− mice. Six weeks after abdominal AC, the consequences of the sustained pressure overload on LV morphology and function were noninvasively and invasively assessed using echocardiography and a 1.4 F conductance catheter. Sham-operated eNOS−/− mice had significantly increased systolic blood pressure, slightly enhanced systolic function (preload recruitable stroke work) and normal diastolic function but no evidence of left ventricular hypertrophy when compared to sham-operated WT animals. AC resulted in a greater increase in anterior wall thickness in eNOS−/− mice (0.8±0.03 mm) compared to WT mice (0.7±0.03 mm; P<0.05). The LV end-diastolic diameter was unchanged by AC in eNOS−/− mice (sham: 3.8±0.1 mm, AC: 3.7±0.2 mm) but significantly increased in WT mice (sham: 3.9±0.1 mm, AC: 4.5±0.2 mm; P<0.05). Interstitial fibrosis and myocyte hypertrophy were greater in eNOS−/− than in WT mice after AC. AC in eNOS−/− mice caused a greater diastolic than systolic dysfunction compared to WT mice.

Conclusion: Chronic pressure overload in eNOS−/− mice results in concentric LV hypertrophy without LV dilation and impaired systolic and diastolic function. These findings suggest that eNOS limits LV remodeling and dysfunction and modulates extracellular matrix proteins under chronic pressure overload.

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Keywords: Heart failure; Left ventricular hypertrophy; Remodeling; Endothelial nitric oxide synthase

1. Introduction

Heart failure evolving in response to sustained hemodynamic overload is among the most prevalent diseases in developed countries [1]. The underlying pathophysiological process, also called remodeling, is divided into two stages. The initial stage is characterized by myocardial hypertrophy as an adaptive response of the heart to increased work load with preserved (compensated) or even enhanced left ventricular systolic function and normal cardiac output [2]. When elevated load persists, the heart evolves to a decompensated state with depressed cardiac function, myocardial fibrosis and increased chamber stiffness [3]. The underlying molecular mechanisms for the initiation and transition from hypertrophy to dilated or restrictive cardiomyopathy are still poorly understood [4].
Nitric oxide (NO) has been implicated in a wide range of physiological functions including endothelium-dependent relaxation of blood vessels, inhibition of platelet aggregation and neutrophil infiltration as well as vascular smooth muscle cell proliferation and migration [5]. NO is formed from L-arginine and oxygen by nitric oxide synthase. The endothelial NO synthase (eNOS) and neuronal NOS (nNOS) are constitutively expressed and can be activated by elevation of intracellular calcium concentrations, whereas the Ca\(^{2+}\)-independent isoform of NOS (iNOS) is usually expressed only in response to cytokines [5]. All three isoforms are expressed in the heart [6].

There has been recent interest in the role of NO in the cardiac contractile function, systemic hypertension as well as left ventricular hypertrophy. Using pharmaceutical tools such as NOS inhibitors or NO releasing substances, e.g. NO-donors, a number of different effects of NO on contractile function have been reported. However, the data in this regard have been controversial. For instance, NO has been shown to have negative inotropic [7], no effect on contractile function [8] or even positive inotropic effects [9]. Interestingly, chronic infusion of the NOS inhibitor N\(^{G}\)-nitro-L-arginine methyl ester (L-NAME) resulted in systemic hypertension with or without the development of left ventricular hypertrophy [10,11]. However, the main limitation of using NOS inhibitors is their only limited selectivity towards one of the three isoforms and, therefore, it is difficult to determine which isoform is responsible for the specific effect. The use of genetically altered animals could overcome these limitations. Indeed, knockout mice for each of the NOS genes have been generated, which allow now to evaluate the specific effect of NO derived from the different isoforms. Interestingly, eNOS-deficient (eNOS\(^{-/-}\)) mice were shown to develop age-related myocardial hypertrophy and have enhanced \(\beta\)-adrenergic inotropic responses [12].

Therefore, in this study, we investigated the specific role of eNOS in pressure-overload induced left ventricular (LV) hypertrophy and compared the morphological and functional changes of the LV between wild-type (WT) and eNOS\(^{-/-}\) mice after abdominal aortic banding.

2. Materials and methods

2.1. Animals

Wild-type C57BL/6 and eNOS\(^{-/-}\) mice were purchased from the Jackson Laboratories (Bar Harbor, Me). eNOS\(^{-/-}\) mice back-crossed to the C57BL/6 background for 8 generations were used in this study. All animals were 2–4 month old when entering the study. Animal experiments were performed in accordance to the German animal protection law and to the guidelines for the use of experimental animals as given by 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).

2.2. Measurement of systolic blood pressure and heart rate

Systolic blood pressure (SBP) and heart rate (HR) were measured using a noninvasive computerized tail-cuff system (BP-2000, Visitech Systems) as previously described [15]. Briefly, mice were trained for 7 days by measuring SBP daily, after which SBP and HR were measured and recorded for 5 consecutive days. Each day, 3 sets of 10 measurements were obtained; a set was accepted if the computer had identified >6 successful readings of 10 measurements.

2.3. Pressure-overload model

Pressure-overload cardiac hypertrophy was produced by abdominal aortic banding. Briefly, mice of both sex weighing 25–32 g (10–14 weeks of age) were anesthetized by intraperitoneal injection of a cocktail of ketamine (50 mg/kg) and xylazine (5 mg/kg). The abdominal aorta was constricted by tightening a 7-0 nylon suture against a blunted 27-gauge needle; the needle was later removed. In control animals, the aorta was isolated but not banded. The incision was then closed, and the animals were allowed to recover for a 6 weeks period. The aortic banding operation resulted in similar trans-stenotic pressure gradients of 47±6 mm Hg in WT and 45±5 mm Hg in eNOS\(^{-/-}\) mice.

2.4. Echocardiography

At baseline, 14 and 42 days after abdominal aortic banding mice were anesthetized with Thiopental (100 mg/kg i.p.), and were allowed to breath spontaneously. The chest was shaved and animals were placed in the left lateral decubitus position on a warming pad to maintain normothermia.

Echocardiograms were performed with an HDI 3000 ultrasonograph (ATL, Solingen, Germany). A dynamically 5–10 MHz linear array transducer was placed on a layer of acoustic coupling gel that was applied to the left hemithorax. Short- and long-axis views of the left ventricle (LV) were obtained by slight angulation and rotation of the transducer. Two-dimensional targeted M-mode studies were obtained at the level of the papillary muscles. Anterior and posterior end-diastolic and end-systolic wall thickness (AWTh and PWh, respectively) and LV internal dimension were measured by using the leading-edge convention of the American Society of Echocardiography. LV fractional shortening (FS) was calculated as: (EDD-ESD)/EDD. The relative wall thickness (h/r) was calculated as: (AWTh+PWh)/EDD and LV mass=1.055 [(AWTh+EDD+PWh)\(^{3}\)-EDD\(^{3}\)], where 1.055 is the specific gravity of the myocardium [12]. Spectral Doppler waveforms were analyzed for peak early and late-diastolic transmural velocities. All measurements
were made from original tracings and three beats were averaged for each measurement.

2.5. Assessment of pressure–volume relationship

At 6 weeks after abdominal aortic banding, mice were anesthetized with thiopental (100 mg/kg i.p.) and Ketamin (50 mg/kg i.m.), intubated and artificially ventilated by a murine ventilator (HUGO SACHS ELEKTRONICS, Freiburg, Germany), with a 1:1 mixture of 100% oxygen and room air and a tidal volume of 10 µl/g BW at 140 breaths/min. The left ventricle was catheterized through the right carotid artery to measure simultaneous pressure–volume relationships, using a miniaturized 1.4 Fr impedance-pressure catheter (Millar Instruments, Houston, Texas). In brief, the method is based on measuring the time-varying electrical conductance of two segments of blood in the left ventricle (LV), from which total volume is calculated. For absolute volume measurements, the catheter was calibrated with known volumes of heparin treated mouse blood, using special calibration cuvettes. Calibrated values were corrected by subtraction of the parallel conductance (conductance of surrounding structures of the cavity), measured by hypertonic saline (15%, 5–10 µl) injection into the right ventricle. Pressure–volume signals were recorded at steady state and during transient preload reduction achieved by transiently occluding the inferior vena cava.

Data were digitized with a sampling rate of 1000 Hz and recorded on a PC using specialized software (HEM, Notocord, Croissy, France). For subsequent analysis of pressure–volume loops, PVAN software (Millar Instruments, Houston, Texas) was used.

2.6. Heart histopathology

The heart tissue was routinely fixed in 4% unbuffered formalin and then prepared according to standard methods. Serial sections were stained with hematoxilin and eosin (H&E)– and elastica van Gieson (EvG). The area of collagen deposition excluding perivascular fibrosis of large vessels was quantitated by a semiautomated imaging analysis (LeicaQWin, Cambridge, GB). Myocyte cross-sectional area was measured from sections stained with H&E, and suitable cross sections were defined as having nearly circular capillary profiles and nuclei. Fifty myocytes from sham-operated and 100 myocytes from mice with AC were analyzed.

2.7. Statistical analysis

All values are given as mean±S.E.M. Student’s unpaired t test was used to compare means between two groups. For comparison of more than two groups, a one-way analysis of variance (ANOVA) was used followed by Dunnett’s post hoc, if appropriate. A P value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Systolic blood pressure and heart rate

First, to confirm that deficiency in eNOS results in systemic hypertension, we measured SBP and HR in eNOS–/– and WT mice. In conscious eNOS–/– mice, SBP was significantly higher when compared to wild-type controls (WT) (124±3 vs. 104±4 mm Hg; P<0.01) confirming the presence of hypertension in eNOS–/– mice, whereas HR was significantly lower in eNOS–/– mice than in WT (565±24 vs. 635±15 bpm; P<0.01).

3.2. Echocardiography and morphometry

LV dimension and function were similar in WT and eNOS–/– before AC as well as 42 days after sham operation (Table 1; Figs. 1 and 3). AC resulted in a progressive increase in LV wall thickness (AWTh, PWTh) in both strains (Table 1; Fig. 2B). However, LV wall thickness was greater in eNOS–/– than in WT mice. In addition, LV weight and LV mass (as calculated by echocardiography) increased to a similar extent in both WT and eNOS–/– mice at 42 days after AC (Table 1; Fig. 2A). In contrast, there was a greater increase in LV/BW weight in eNOS–/– (+66%) compared to WT (+46%) mice (P<0.05). Sustained pressure-overload resulted in WT mice in LV dilatation at day 42 whereas end-diastolic and end-systolic dimension (EDD and ESD) were not different between sham-operated and aortic constricted eNOS–/– mice (Fig. 1). Consequently, the ratio of LV wall thickness to LV radius (relative wall thickness) significantly increased in eNOS–/– mice with AC but not in WT mice (Fig. 2). At day 42 after AC, fractional shortening (FS) and velocity of circumferential fiber shortening (vcf) were similarly reduced in WT and eNOS–/– mice (Table 1; Fig. 2). In WT mice, peak E and A velocity as well as the E/A ratio were not different between sham-operated and AC animals (Table 1). In contrast, chronic pressure-overload resulted in a significant increase in peak E velocity and a decrease in peak A velocity in eNOS–/– mice. Consequently, the E/A ratio significantly increased indicating restrictive diastolic filling pattern in eNOS–/– mice with AC (Table 1).

M-mode echocardiography from the parasternal short-axis view of a WT and an eNOS–/– mouse at 42 days after pressure-overload is illustrated in Fig. 1.

3.3. Hemodynamic measurements

Cardiac function was assessed in vivo by using a miniaturized 1.4F conductance catheter which simultaneously measures pressure and volume. End-systolic and end-diastolic volumes (ESV and EDV) as well as ejection fraction (EF) and the cardiac output index (COI) were similar in sham-operated WT and eNOS–/– mice.
In contrast, systolic function was significantly enhanced in sham-operated eNOS\(^{-/-}\) mice when compared to sham-operated WT mice as indicated by an increase in end-systolic pressure (ESP), SW index (SWI), as well as by the load-independent parameters preload recruitable stroke work (PRSW), end-systolic volume elastance (\(E_{es}\)) and the slope of the end-systolic pressure–volume relationship (ESPVR) (Table 2; Fig. 3). Peripheral vascular resistance was increased in sham-operated eNOS\(^{-/-}\) mice, since the arterial elastance (\(E_a\)) was significantly higher when compared to sham-operated WT controls. The \(E_a/E_{es}\) ratio was also elevated (\(>1\)) in sham-operated eNOS\(^{-/-}\) mice, indicating uncoupling of the LV to the vasculature (Table 2). Rate of decay of pressure (dP/dt\(_{min}\)), the mono-exponential time constant of relaxation (\(\tau\)) and the end-diastolic pressure volume relationship (EDPVR) as means of diastolic function tended to be higher in sham-operated eNOS\(^{-/-}\) and WT mice (Table 2; Fig. 3C,E).

AC for 6 weeks resulted in a significant increase in end-systolic and end-diastolic volume and pressure (ESP, EDP, ESV and EDV) in WT mice whereas in eNOS\(^{-/-}\) mice the ESP decreased and EDP increased. The ESV remained unchanged in eNOS\(^{-/-}\) with AC whereas the EDV tended to being decreased when compared to sham-operated animals but were significantly reduced when compared to WT mice with AC. Moreover, EF, SWI and COI significantly decreased in both WT and eNOS\(^{-/-}\) mice with AC (Table 2). Systolic LV function was significantly impaired after AC

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Echocardiographic and morphometric data</th>
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<tbody>
<tr>
<td></td>
<td>Wild-type</td>
</tr>
<tr>
<td></td>
<td>Sham ((n=8))</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>28.3±0.5</td>
</tr>
<tr>
<td>wwt LV, mg</td>
<td>107±6</td>
</tr>
<tr>
<td>wwt LV /body weight</td>
<td>3.67±0.22</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>433±22</td>
</tr>
<tr>
<td>AWTTh, mm</td>
<td>0.52±0.01</td>
</tr>
<tr>
<td>PWTh, mm</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>36.2±1.5</td>
</tr>
<tr>
<td>Peak E velocity, cm/s</td>
<td>42.2±2.6</td>
</tr>
<tr>
<td>Peak A velocity, cm/s</td>
<td>19.7±1.2</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>2.0±0.1</td>
</tr>
</tbody>
</table>

wwt LV: left ventricular wet weight; AWTTh: anterior wall thickness; PWTh: posterior wall thickness. \(n=8–10\)/group.

\(^a\) \(P<0.05\) for WT vs. eNOS.

\(^*\) \(P<0.05\) vs. preband.

Fig. 1. Effect of chronic pressure overload on left ventricular dimension and systolic function. Representative M-mode echocardiograms obtained from wild-type (WT, A) and eNOS\(^{-/-}\) (B) mice at day 42 after aortic banding (AB). (C–E) Echocardiographic measurements of end-diastolic dimension (EDD), velocity of circumferential fiber shortening (vcf), a measurement of contractility and end-systolic dimension (ESD) in WT and eNOS\(^{-/-}\) mice after sham operation (S) or AB. Values are mean±S.E.M. \(n=7–8\) per group. *\(P<0.05\) vs. S.
in WT mice as indicated by a decrease in dP/dt_{\text{max}} (−33%), E_{\text{es}} (−32%), PRSW (−60%) and ESPVR. In eNOS−/− mice, LV systolic function was even tended to be even more depressed (dP/dt_{\text{max}}: −42%, E_{\text{es}}: −61% (P<0.05 vs. WT), PRSW: −57%). Moreover, E_{\text{a}} and the E_{\text{a}}/E_{\text{es}} ratio increased in both group after AC but even higher in eNOS−/− compared to WT mice with AC (Table 2; Fig. 3). Diastolic function was impaired in WT mice after AC as indicated by a decrease in dP/dt_{\text{min}} (−20%) and an increase in τ (+61%) and EDPVR (+81%). The decrease in diastolic function after AC was even more pronounced in eNOS−/− mice (dP/dt_{\text{min}}: −39%, EDPVR: +115%, both P<0.05 vs. WT; τ +61%) (Table 2; Fig. 3D,F).

3.4. Heart histopathology

In WT mice, AC for 6 weeks resulted in the formation of a patchy interstitial fibrosis with a reticular pattern which was far more prominent in the respective eNOS−/− mice (Fig. 4). In contrast, sham-operated WT and eNOS−/− mice displayed no fibrosis. Quantitative analysis of the left ventricular volume fraction confirmed the increase in

Table 2
Hemodynamics of eNOS−/− and WT mice 42 days after aortic banding or sham operation based on in vivo analysis of pressure–volume relationship

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-type</th>
<th>eNOS−/−</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Aortic banding (n=8)</td>
<td>Aortic banding (n=8)</td>
</tr>
<tr>
<td></td>
<td>Sham (n=7)</td>
<td></td>
</tr>
<tr>
<td>Hemodynamics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESP (mm Hg)</td>
<td>94 ± 4*</td>
<td>110 ± 5*</td>
</tr>
<tr>
<td>EDP (mm Hg)</td>
<td>2.2 ± 0.6</td>
<td>4.7 ± 0.8*</td>
</tr>
<tr>
<td>ESV (µl)</td>
<td>16 ± 3</td>
<td>40 ± 6*</td>
</tr>
<tr>
<td>EDV (µl)</td>
<td>36 ± 1</td>
<td>54 ± 5*</td>
</tr>
<tr>
<td>EF (%)</td>
<td>58 ± 4</td>
<td>27 ± 4*</td>
</tr>
<tr>
<td>SWI (mm Hg* µl/mg)</td>
<td>14.3 ± 2</td>
<td>8.2 ± 1</td>
</tr>
<tr>
<td>COI (µl/min/g)</td>
<td>349 ± 41</td>
<td>230 ± 35*</td>
</tr>
<tr>
<td>Systolic function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dP/dt_{\text{max}} (mm Hg/s)</td>
<td>8950 ± 543</td>
<td>5962 ± 476*</td>
</tr>
<tr>
<td>E_{\text{a}} (mm Hg/µl)</td>
<td>3.5 ± 0.4</td>
<td>7.9 ± 0.9*</td>
</tr>
<tr>
<td>E_{\text{es}} (mm Hg/µl)</td>
<td>3.1 ± 0.3</td>
<td>2.1 ± 0.6*</td>
</tr>
<tr>
<td>E_{\text{a}}/E_{\text{es}}</td>
<td>0.9 ± 0.1</td>
<td>3.4 ± 0.07*</td>
</tr>
<tr>
<td>PRSW (mm Hg)</td>
<td>83 ± 4*</td>
<td>32 ± 6*</td>
</tr>
<tr>
<td>Diastolic function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dP/dt_{\text{min}} (mm Hg/s)</td>
<td>−8575 ± 539</td>
<td>−6907 ± 195</td>
</tr>
<tr>
<td>τ (ms)</td>
<td>6.5 ± 0.2</td>
<td>10.5 ± 0.4*</td>
</tr>
<tr>
<td>EDPVR (mm Hg/µl)</td>
<td>0.017 ± 0.004</td>
<td>0.031 ± 0.005*</td>
</tr>
</tbody>
</table>

ESP: end-systolic pressure; EDP: end-diastolic pressure; ESV: end-systolic volume; EDV: end-diastolic volume; COI: cardiac output index; dP/dt_{\text{max}}: maximal rate of pressure development; dP/dt_{\text{min}}: maximal rate of decay of pressure; EF: ejection fraction; SW: stroke work; SWI: stroke work index; E_{\text{es}}: arterial elastance; E_{\text{a}}: end-systolic volume elastance; PRSW: preload recruitable stroke work; τ: monoexponential time constant of relaxation; EDPVR: end-diastolic pressure volume relationship.

* P<0.05 when compared wild-type vs. appropriate eNOS.

† P<0.05 when compared to appropriate sham.
interstitial fibrosis in eNOS−/− with AC compared to the respective WT mice (Fig. 4). AC led to a significant increase in myocyte size in WT mice which was further increased in eNOS−/− mice (P<0.05; Fig. 4).

4. Discussion

The results of this study emphasize that eNOS is not only involved in blood pressure regulation but also plays a fundamental role in LV remodeling under chronic pressure overload. Indeed, chronic pressure-overload in eNOS−/− mice induced by abdominal aortic banding results in severe restrictive cardiomyopathy phenotype with the following features: (i) concentric left ventricular hypertrophy with marked increase in relative wall thickness, (ii) smaller systolic and diastolic dimensions, (iii) marked interstitial fibrosis, (iv) depression of systolic contractility and (v) impaired diastolic relaxation with elevated chamber stiffness and restrictive filling pattern. Moreover, eNOS−/− mice at baseline had higher systolic blood pressure due to increased arterial elastance ($E_a$), stressing...
the role of eNOS in maintaining vascular tone, and enhanced basal contractility. The $\frac{E_a}{E_es}$ ratio was already slightly but significantly elevated indicating uncoupling of the LV to the vasculature. Interestingly, stiffening of the arterial wall and a reduction in peripheral vasomotor regulation can profoundly affect the vascular–ventricular coupling by imposing far greater pulsatile and late-systolic load on the heart. This is accompanied by increases in left ventricular end-systolic stiffness and reduced diastolic compliance [13]. Indeed, the end-systolic volume elastance ($E_{es}$) and the time constant of isovolumic relaxation ($\tau$) was significantly increased when compared to WT mice. Although blood pressure and LV systolic function were increased in eNOS−/− mice, there was no evidence of LV hypertrophy at the age of 4–5 month, confirming previous reports [14].

The enhanced basal contractility in eNOS−/− mice seen in our study is in disagreement with previous findings showing that baseline contractility (dP/dt$_{max}$) as assessed using tip catheters was not different between WT and eNOS−/− mice [15,16]. Importantly, we used in the present study a 1.4 F Millar pressure–volume catheter which allows the assessment of load-dependent (including dP/dt$_{max}$) as well as of load-independent parameters such as PRSW, ESPVR and $E_{es}$. Indeed, in eNOS−/− mice ESPVR, $E_{es}$ and particularly PRSW, which is (i) a modification of the Frank–Starling law, (ii) less influenced by noise or ventricular geometry, (iii) linear and (iv) preload independent and afterload insensitive over the physiological range [17], were significantly increased when compared to WT mice emphasizing the presence of hypercontractility in eNOS−/− mice. Hypercontractility has also been reported in hypertensive patients [1] as well as in spontaneously hypertensive rats [18].

Chronic pressure overload by abdominal AC resulted in LV hypertrophy in both eNOS−/− and WT mice. Interestingly, hearts from WT mice were characterized by eccentric LV remodeling with increase in wall thickness, LV dilation but no change in relative wall thickness (ratio of LV wall thickness to LV radius). In contrast, eNOS−/− mice
exhibited concentric LV remodeling with marked increase in relative wall thickness which was due to increased LV wall thickness without a change in ESD and EDD as well as marked interstitial fibrosis. Concentric LV remodeling has also been observed in 14–20 months old eNOS−/− [12] suggesting that abdominal AC accelerates this unique age-related phenotype. Moreover, in a mouse model of heart failure induced by myocardial infarction, eNOS−/− mice had an increased LV mass which was associated with an increase in myocyte diameter when compared to WT mice [15]. The increase in LV mass after myocardial infarction in eNOS−/− mice was also seen when elevated blood pressure was normalized by hydralazine. In these mice, the beneficial effects of ACE inhibitor and AT1 antagonist therapy on LV remodeling were substantially attenuated [14] suggesting that an increase in cardiac eNOS activity contributes to the cardioprotective effects of these established pharmacologic interventions. In addition, eNOS−/− mice with chronic pressure-overload induced by transverse aortic constriction (TAC), a renin-angiotensin system (RAS) independent model, also exhibit enhanced LV hypertrophy, systolic and diastolic function and increased fibrosis, all of which persisted even when blood pressure was normalized by hydralazine [16]. In contrast to the present study, TAC caused eccentric LV hypertrophy in eNOS−/−, but not in WT mice, which was accompanied by LV systolic and diastolic dysfunction. There are several reasons which may explain the differences between the findings from both studies. Most importantly, TAC is a more severe model of chronic pressure-overload as indicated by a trans-stenotic pressure gradient of ~90 mm Hg [16], in contrast to ~45 mm Hg in this study. Moreover, TAC is, in contrast to abdominal AC, a RAS-independent model which may have affected the phenotype in eNOS−/− mice with pressure-overload. Indeed, LV hypertrophy induced by abdominal AC is due to an activation of the circulating and local RAS [20]. Of note, locally produced Ang II (Ang II), more than circulating Ang II, is a very potent direct stimulator of cardiomyocyte hypertrophy and fibrosis without an increase in EDPVR as assessed by conductance catheter. These findings are characteristic for increased diastolic chamber stiffness closely resembling.

However, for the in vivo situation, it remains unclear whether eNOS expressed by endothelial cells from the coronary microcirculation or from cardiac myocytes is predominantly responsible for the effects on LV remodeling [25]. Interestingly, cardiomyocyte-restricted overexpression of NOS3 limits LV dysfunction and remodeling after MI and attenuates LV hypertrophy induced by chronic isoproterenol infusion [26,27] suggesting that NO derived from eNOS in cardiac myocytes may at least in part contribute to the beneficial effect on LV remodeling in an autocrine manner. The anti-hypertrophic and anti-proliferative effects of NO are partially due to the formation of cGMP via activation of the soluble guanylate cyclase (sGC) [28]. Noteworthy, mice which are deficient for natriuretic peptide receptor A, a particulate GC, develop cardiac hypertrophy and fibrosis [29] as well as marked LV dysfunction after TAC [30]. Together, this suggests that NO derived from eNOS attenuates cardiac hypertrophy and fibrosis induced by chronic pressure-overload which is probably in part mediated by cGMP.

The morphological changes of the hearts after chronic pressure overload were accompanied by systolic and diastolic dysfunction and deterioration of ejection performance in both WT and eNOS−/− mice. Indeed, the load-dependent parameters EF, SW, SWI, COI and dP/dtmax as well as the load-independent parameter $E_{cs}$ and PRSW decreased when compared to sham-operated animals revealing depressed contractile function. Interestingly, in aortic banded WT mice $E_a$ significantly increased, as expected, whereas in eNOS−/− the elevated $E_a$ did not further increase due to AC suggesting that the deterioration of ejection performance in aortic banded eNOS−/− mice is due to changes in the left ventricle rather than in the vasculature. Indeed, chronic pressure-overload in eNOS−/− mice resulted in a massive interstitial fibrosis which was accompanied by a dramatically decrease in ventricular elastance ($E_{es}$). Consequently, the ventricular-to-vascular coupling ratio ($E_a/E_{es}$) was significantly increased in eNOS−/− with AC when compared to sham-operated animals but also to WT mice with AC. In normal human hearts, $E_a/E_{es}$ is ~0.5 [13], and in patients with depressed left ventricular function, the ratio has been reported to be >1. It has been suggested that the increase in $E_a/E_{es}$ from 0.5 is associated with a decrease in mechanical efficacy [17].

Diastolic function was also impaired in both WT and eNOS−/− mice with AC as indicated by an increase in the time constant of isovolumic relaxation ($\tau$) and a depression of $dP/dt_{min}$. In addition to a depression in diastolic relaxation, eNOS−/− mice also showed a diastolic filling pattern which was characterized by an increased peak E-wave velocity and E/A ratio measured by echocardiography as well as an increase in EDPVR as assessed by conductance catheter. These findings are characteristic for increased diastolic chamber stiffness closely resembling.
hypertensive hypertrophic cardiomyopathy with increased myocardial fibrosis of the elderly in humans [13]. Indeed, AC in eNOS−/− mice was associated with enhanced myocardial hypertrophy and increased interstitial fibrosis when compared to WT mice. Interestingly, in eNOS−/− mice with heart failure diastolic dysfunction was also more marked when compared to WT mice [15]. Moreover, inhibition of basal NO production by the NOS inhibitor L-NMMA significantly impaired left ventricular diastolic filling [31]. In contrast, exogenous NO improves left ventricular relaxation in isolated ferret papillary muscle preparation [32] and in normal human subjects [33]. Thus, it is conceivable that NO derived from eNOS contributes to myocardial relaxation and suppresses the development of interstitial fibrosis.

In summary, we demonstrated that eNOS−/− mice are not only hypertensive but are also characterized by enhanced systolic function and depressed diastolic function without left ventricular hypertrophy when compared to WT animals. Induction of chronic pressure overload exhibited concentric LV hypertrophy without dilation in hearts from eNOS−/− mice which was associated with a massive interstitial fibrosis, whereas hearts from WT mice developed eccentric LV hypertrophy. These morphological changes were accompanied by systolic and diastolic dysfunction and deterioration of ejection performance in both WT and eNOS−/− hearts. These findings suggest that eNOS limits LV remodeling and dysfunction and modulates extracellular matrix proteins under chronic pressure-overload.

References

