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Tissue Inhibitor of Matrix Metalloproteinase-1 in Norepinephrine-Induced Remodeling of the Mouse Heart

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Key Words

Adrenergic agonists • Extracellular matrix • Hypertrophy • Matrix metalloproteinases • Ventricular remodeling

Abstract

Background: Matrix metalloproteinases (MMPs) play an important role in myocardial remodeling. Their activity is regulated by the tissue inhibitors of metalloproteinases (TIMPs). The present study analyzed the contribution of changes in functional and molecular parameters to early cardiac remodeling in mice hearts. The role that TIMPs might play in this process was specially acknowledged. Methods: The remodeling was induced by norepinephrine (NE) given sc in balb/c mice. Varying concentrations, time and the addition of a neutralizing TIMP-1 antibody were evaluated. Results: High dose NE led to insufficiency of the left ventricle (LV) as evidenced by reduced NE-induced elevation of LV systolic pressure, contractility and relaxation. Further, signs of lung congestion were seen. NE induced a concentration-dependent increase of LV weight/body weight (LVW/BW) ratio and elevated mRNA expression of atrial natriuretic peptide (ANP). This was accompanied by induction of collagen type I and III, as well as TIMP-1 expression. Conclusions: The NE-induced increase of TIMP-1 expression may induce the elevation of the antihypertrophic cardiac factor ANP since NE-induced increase of ANP expression was abolished by neutralizing TIMP-1 antibody. Thus, TIMP-1 may mediate ANP-induced attenuation of NE-induced hypertrophy in the mouse heart.

Introduction

Cardiac remodeling is accompanied by changes in the cellular constituents of the LV myocardium with a significant alteration of the structure and composition of the extracellular matrix (ECM) [1, 2]. Moreover, it has become increasingly evident that myocardial ECM is not

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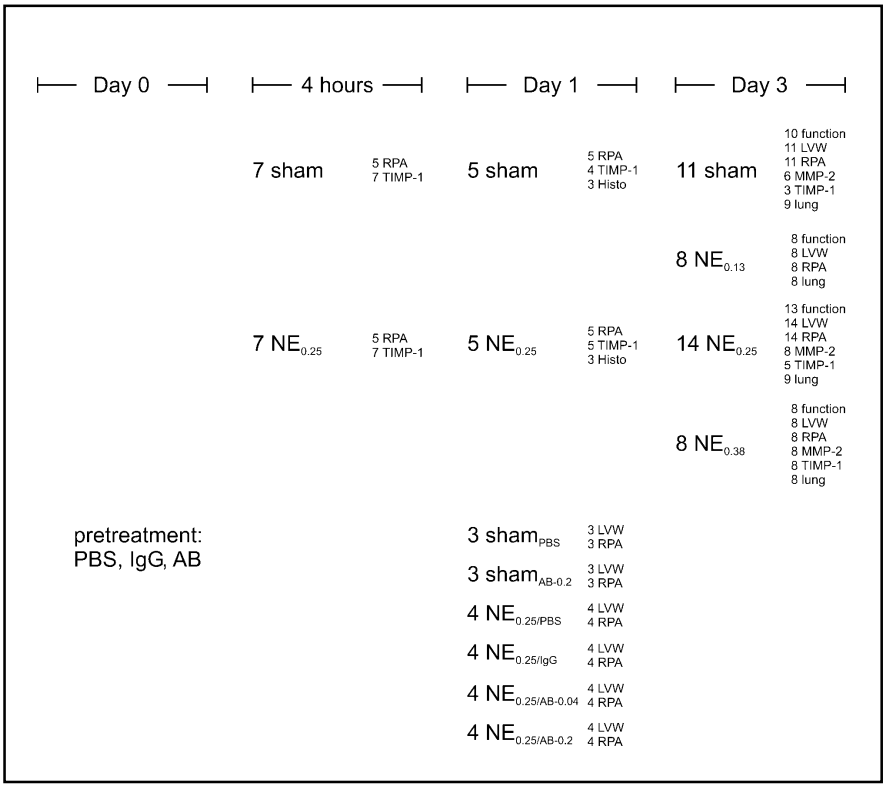

Fig. 1. Design of the protocol, with number of animals receiving norepinephrine (NE, 0.13, 0.25 or 0.38 mg/kg per h, respectively), PBS, neutralizing anti-TIMP-1 antibody (AB; 0.2 mg/kg per h) or combination of NE and PBS, IgG (0.2 mg/kg per h) and AB (0.2 or 0.04 mg/kg per h, respectively) for various periods of time (4 hours, 1 day or 3 days). Corresponding measurements are indicated: RNA preparation and ribonuclease protection assay (RPA); TIMP-1 ELISA (TIMP-1); immunohistochemical detection of TIMP-1 (Histo); determination of the left ventricular weight (LVW); hemodynamic measurement (function); detection of MMP-2 activity (MMP-2); determination of lung weight and histology (lung).

a static structure, but rather a dynamic entity that may play a fundamental role in myocardial adaptation to pathologic stress and thereby facilitate the remodeling process [2, 3]. The remodeling of ECM is characterized by an elevated turnover of the matrix proteins caused by elevated activity of matrix metalloproteinases (MMPs). The level of MMP synthesis is an important determinant of matrix degradation, but the true degradative capacity of the MMPs depends on the activation of the MMP proenzyme. The MMPs after being synthesized in a latent, proenzyme, or zymogen form, are secreted into the extracellular space. An important control of MMP activity is the inhibition of the activated enzyme by action of a group of specific MMP inhibitors called tissue inhibitors of matrix metalloproteinases (TIMPs) [3].

Norepinephrine (NE) is well known to cause LV hypertrophy in rats [4]. LV hypertrophy is characterized by increased myocyte diameter and elevation of relative heart weight accompanied by increase of atrial natriuretic peptide (ANP) [4]. ANP is part of the program of fetal gene induction, a hallmark of cardiac hypertrophy [5]. The development of LV hypertrophy is accompanied by increased collagen expression [6] and elevated MMP activity [7]. The purpose of this study was to investigate the possible contribution of ECM turnover to early cardiac remodeling in mice. Therefore, LV hypertrophy was detected by measuring the relative heart weight and the expression of natriuretic peptides. ECM remodeling was characterized by the analysis of the expression of collagen I and III, the main components of the ECM, mRNA expression and the activity of MMPs and TIMPs. In addition, heart function was measured and the lung was analyzed histological for possible manifestations of the consequences of heart failure. A strong NE-induced increase of TIMP-1 was not expected. The role of TIMP-1 in NE-induced early remodeling was analyzed by application of a neutralizing anti TIMP-1 antibody.

Material and Methods

Animal Experiments

Male BALB/c mice (purchased from Charles River, Sulzfeld, Germany; n = 88; weight: 24.2±0.22 g; age: 11.0±0.11 weeks) were used. Norepinephrine (NE) (Sigma, Deisenhofen, Germany) was dissolved in 0.9% NaCl with 100 mg/L ascorbic acid (Merck, Darmstadt, Germany) and administered at a dose of 0.13, 0.25 and 0.38 mg/kg per h, respectively, by Alzet mini-osmotic pumps (Alza, Paolo Alto, CA, USA) placed subcutaneously for 3 days. The dose range of NE was chosen on the base of rat experiments with intra venous application of 0.1 mg/kg per h [8]. Animals with an unfilled mini-osmotic pump re-

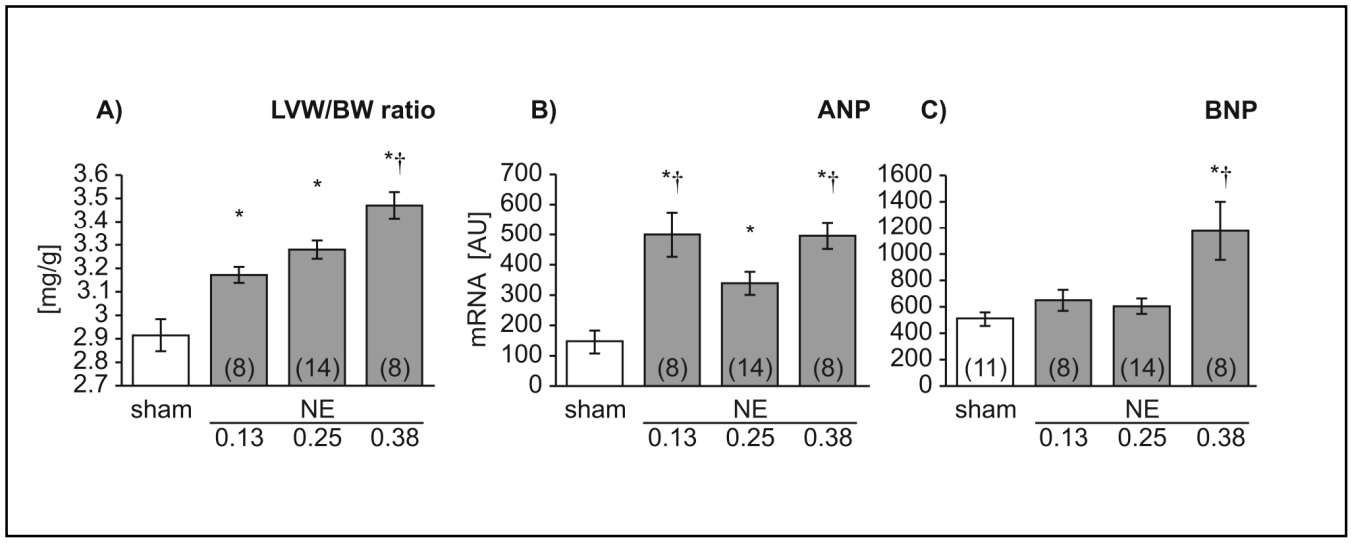

Fig. 2. Effect of increasing concentration of norepinephrine (NE) on the left ventricular weight/body weight (LVW/BW) ratio (A) and on atrial (ANP) (B) and brain natriuretic peptide (BNP) expression (C) from mice. The results of NE-treatment (0.13, 0.25 and 0.38 mg/kg per h, respectively) for 3 days were compared with sham operated controls. Relative abundance of mRNA expression obtained by RPA detection with 2.5 µg of total RNA was related to GAPDH mRNA expression ($x10^{-3}$) as arbitrary units (AU). Values are means ± SEM. *P<0.05 vs. sham; †P<0.05 vs. NE 0.25; number of measurements in parentheses.

placement served as controls. To evaluate the time course of the NE on TIMP expression NE was administered in a second experiment at a dose of 0.25 mg/kg per h for 4 hours or 1, and 3 days, respectively. In the third experiment the effect of the neutralizing TIMP-1 antibody (AB) was elucidated. The goat anti-mouse TIMP-1 AB (R&D Systems, Wiesbaden, Germany), PBS, or goat IgG (R&D Systems) were injected subcutaneously twice one day before treatment and during the pump implantation. The neutralizing activity of the AB was measured by the inhibition of rmMMP-2 *in vitro*. The AB (0.2 and 0.04 mg/kg per h, respectively) and IgG (0.2 mg/kg per) was reconstituted in PBS and 50 µL were injected. The study groups are summarized in Fig. 1. Animal care and use were in accordance with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the appropriate State agency of Saxony. The animals were allowed to move freely in their cages with access to tap water and standard chow diet ad libitum.

Hemodynamic measurements

Heart function was measured with ultra miniature catheter pressure transducers in closed-chest spontaneously breathing animals anaesthetized with thiopental sodium (Trapanal® 80 mg/kg i.p., Byk Gulden, Konstanz, Germany) as previously described [9].

Sample preparation

Animals were anesthetized (Trapanal® 100 mg/kg i.p., Byk Gulden). Hearts were rapidly excised either immediately or after hemodynamic measurement. The right ventricle (RV) was trimmed away and the left ventricle (LV) was weighed and frozen in liquid nitrogen. The lung was excised, weighed, divided, and one part was fixed in 4% buffered formaldehyde, and the other part was frozen in liquid nitrogen. Lung specimens of 34 animals were dried for 10 hours at 95°C. In these lung samples, the wet-to-dry weight ratio was determined as an indicator of potential edema.

Histological analysis

The LV from animals treated for 1 day was weighed, and transverse sliced. The basal part of the heart was frozen in liquid nitrogen. The apical part of the LV was fixed in 4% buffered formaldehyde. For immunohistochemical analysis the formalin-fixed part of LV was embedded in Paraplast (Sigma), cut into 7 µm sections and mounted on slides. Immunostaining was performed using the Cell and Tissue Staining Kit (HRP-AEC system, R&D Systems) as described by the manufacturer. Goat anti-mouse TIMP-1 AB (R&D Systems) was used as primary antibody (0.1 µg/mL) and incubated for 1 h at 37°C. After immunostaining the slices were counterstained with hematoxylin for 1 min and mounted in a drop of aqueous mounting medium (R&D Systems). For negative control staining the primary AB was omitted.

The formalin-fixed and paraffin-embedded lung samples were sectioned into 7 µm slices and stained with hematoxylin and eosin (H&E).

RNase protection assay (RPA)

The mRNA of ECM proteins, of natriuretic peptides, of MMP and TIMP was detected by the RNase protection assay (RPA) as previously described [10].

	sham	NE		
		0.13	0.25	0.38
	(n = 10)	(n = 8)	(n = 13)	(n = 8)
Heart rate, bpm	416 ± 11.8	631 ± 34.1*	615 ± 35.4*	650 ± 35.4*
LV systolic pressure, mmHg	110 ± 3.9	150 ± 3.9*	133 ± 4.4*†	132 ± 5.3*†
LV end-diastolic pressure, mmHg	4.6 ± 0.8	1.9 ± 0.5*	4.0 ± 0.4	2.4 ± 0.6*
Diastolic aortic pressure, mmHg	78 ± 3.4	103 ± 4.3*	91 ± 5.5*	93 ± 4.2*
LVdP/dt_{max}, mmHg/s	8670 ± 786.8	19745 ± 632.8*	14407 ± 1211*†	14767 ± 876.2*†
LVdP/dt_{min}, mmHg/s	7902 ± 632.1	13011 ± 560.5*	9358 ± 513.2*†	9453 ± 483.3*†

Table 1. Changes in functional parameters of the left ventricle (LV) in mice after 3 days of different concentration of norepinephrine (NE; 0.13, 0.25, 0.39 mg/kg·h, s.c., respectively) treatment. Animals with an unfilled mini-osmotic pump replacement for 3 days served as controls (sham). Values are mean ± SEM.;* $P < 0.05$ vs. corresponding sham; † $P < 0.05$ vs. 0.13 NE; number of measurements in parentheses.

MMP-2 activity

The Biotrak MMP-2 activity assay system (Amersham Biosciences, Freiburg, Germany) provides quantitative determination of MMP-2 activity in the cardiac tissue after homogenization [8].

TIMP-1 ELISA

An Enzyme-Linked Immuno-Sorbent Assay (ELISA, R&D Systems) was used for the determination of mouse TIMP-1 in 50 µL of the cardiac tissue, which was used for MMP-2 activity determination (detection range: 37.5-2400 pg/mL). Incubation and further procedures were performed according to the manufacturer's instruction. The absorbance was measured at 450 nm with wavelength correction at 570 nm using a Spectra count (Packard Instruments, Illinois, USA). TIMP-1 concentrations were calculated using the program I-smart (Packard Instruments) with a 4-parameter-regression curve fitting.

Statistical analysis

All data were analyzed and expressed as mean ± SEM. A multiple-sample comparison (ANOVA and multiple range tests using the criterion of the least significant differences) was applied to test the differences between the groups. Different modes and time periods of treatment were proven for significance. A value of $p<0.05$ was considered to be significant.

Results

LV hypertrophy

NE increased the LV weight/body weight (LVW/BW)-ratio (Fig. 2A) in a dose dependent manner. The expression of ANP was maximal increased already with the lowest NE dose (Fig. 2B) after 3 days of treatment. The mRNA expression of another natriuretic peptide, the brain natriuretic peptide (BNP), was elevated only at high dose of NE (Fig. 2C). The C type of natriuretic peptides (CNP) was not detectable with 2.5 µg of total RNA.

Heart function

The measurements of LV function revealed an elevation of heart rate (HR), LV systolic pressure (LVSP), diastolic aortic pressure (DAP) as well as LV contractility (LV dP/dt_{max}) and LV relaxation (LV dP/dt_{min}) in mice after 3 days of NE treatment (Tab. 1). LV end-diastolic pressure was decreased by NE-stimulation at least by low and high dose NE. The elevation of LVSP, as well as of LV dP/dt_{max} and LV dP/dt_{min}, was more pronounced with the lowest NE concentration.

Lung

The relative lung weight was increased in a concentration dependent manner of NE (Fig. 3A). This was accompanied by an increase of tissue substance, since the wet-to-dry weight ratio was decreased by the highest dose of NE (Fig. 3B). In addition, there was pronounced hemorrhage which occurred predominantly with high dose NE (Fig. 3D). This hemorrhage was already seen macroscopically as red spots in the freshly prepared lung.

Extracellular matrix

Sustained activation of metabolism of the extracellular matrix (ECM) was indicated by the concentration-

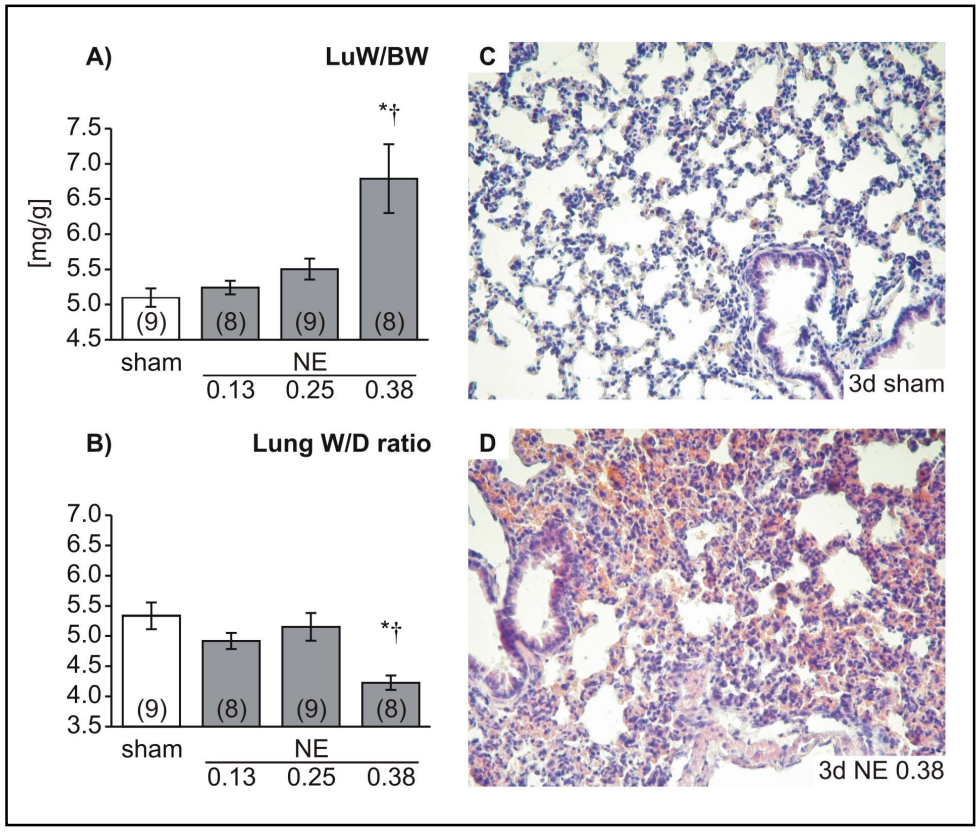

Fig. 3. Changes of lung weight/body weight ratio (LuW/BW) (A), wet-to-dry weight ratio of the lung (Lung W/D ratio) (B) and histological changes of the lung (C-D) in norepinephrine (NE)-treated mice. The results of NE-treatment (0.13, 0.25 and 0.38 mg/kg per h, respectively for 3 days) were compared with sham operated controls. Means ± SEM.; *P < 0.05 vs. sham; †P<0.05 vs. NE 0.25; number of measurements in parentheses. Histological changes of the lung in NE-treated mice were shown by representative hematoxylin-eosin staining in lung section of a mice treated 3 days with NE (0.38 mg/kg per h) (D) in comparison to a lung section of a sham operated control (C) (original magnification 36x).

dependent increase in the mRNA expression of colligin, collagen type I and type III (Fig. 4A-C, respectively) after 3 days of NE-treatment in the LV. The elevation of collagen type I as well as type III was clearly more pronounced with high dose NE (Fig. 4B and C, respectively). The ratio of type III/type I collagen was increased in a concentration dependent manner (Fig. 4D). However, with high dose NE (0.38 mg/kg per h) the ratio was not different from sham one.

The expression of the TIMP-2, -3 and -4 was not changed (Fig. 5A, Tab. 2). While MMP-2 expression was higher than MMP-9 expression (Fig. 5A, Tab. 2), neither MMP-2 nor MMP-9 levels were changed by NE administration (Tab. 2). Both collagenases of the mouse (MMP-8 and MMP-13) were not detectable with 7.5 µg of total RNA. The mRNA expression of TIMP-1 (Fig. 5B) and the activity of MMP-2 (Fig. 5C) was increased after 3 days of NE-treatment, although the MMP-2 mRNA expression was not changed (Fig. 5A). In contrast to the concentration dependent increase of the mRNA expression of TIMP-1 (Fig. 5B), the activity of MMP-2 did not change with a higher NE dose (Fig. 5C).

Since the stoichiometry between the gene expression of MMPs and TIMPs takes part in the regulation of remodeling of the extracellular matrix, we calculated the ratios of MMP-2 to TIMPs [11]. The concentration dependent decrease of the MMP-2/TIMP-1 ratio (Tab. 2) was obvious after the strong increase of TIMP-1 after increasing concentrations of NE (Fig. 5B). This decrease was detectable on RNA level comparing mRNA expression of MMP-2/TIMP-1 as well as on protein level comparing the activity of MMP-2 and the protein concentration of TIMP-1 (Tab. 2). There was a strong correlation of both parameters (Fig. 5D). However, the decrease of the MMP-2/TIMP-1 ratio did not explain the NE-induced increase of the MMP-2 activity (Fig. 5C). The NE-induced increase of the MMP-2/total TIMP ratio (Fig. 5E) was comparable to NE-induced increase of the MMP-2 activity (Fig. 5C). Both were increased with 0.25 mg/kg per h NE and not further elevated with higher concentration of NE. The MMP-2/TIMP-2 ratio was not affected (Tab. 2). The MMP-2/TIMP-3 ratio was increased in a concentration dependent manner and the MMP-2/TIMP-4 ratio was increased with low dose NE (Tab. 2).

Changes in the expression of TIMPs in the left ventricle

To further elucidate why TIMP-1 mRNA expression was elevated despite elevated MMP-2 activity (Fig. 5C), the time-course of TIMP mRNA expression was

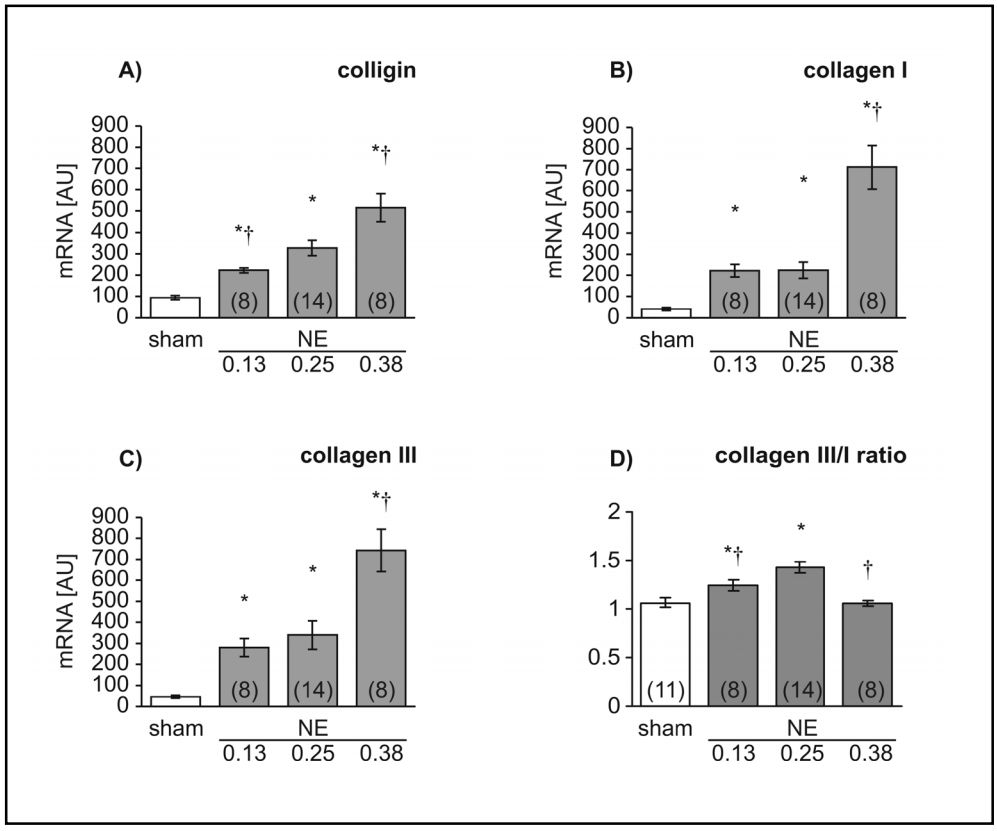

Fig. 4. Changes in the expression of extracellular matrix proteins after treatment with increasing concentrations of norepinephrine (NE) for 3 days in the left ventricle of mice. Colligin (A), collagen I (B) and collagen III (C) mRNA expression in the left ventricle from sham operated controls and mice treated 3 days with increasing concentrations of NE (0.13, 0.25 and 0.38 mg/kg per h, respectively) were analyzed by RPA detection. Relative abundance of mRNA expression with 2.5 µg of total RNA was related to GAPDH mRNA expression ($x10^{-3}$) as arbitrary units (AU). Values are means ± SEM. *P<0.05 vs. sham; †P<0.05 vs. NE 0.25; number of measurements in parentheses.

	sham	NE		
		0.13	0.25	0.38
	(n=11)	(n=8)	(n=14)	(n=8)
MMP-2, AU	6.84 ± 0.74	8.38 ± 1.49	10.78 ± 2.02	9.94 ± 1.43
MMP-9, AU	0.17 ± 0.02	0.11 ± 0.02	0.16 ± 0.02	0.17 ± 0.05
TIMP-1, AU	0.76 ± 0.09	2.74 ± 0.36	10.98 ± 4.76	23.89 ± 5.95*†
TIMP-2, AU	31.77 ± 5.36	35.09 ± 7.44	47.41 ± 9.81	39.03 ± 7.99
TIMP-3, AU	59.94 ± 12.42	45.28 ± 5.50	40.33 ± 6.27	27.98 ± 3.48
TIMP-4, AU	4.84 ± 0.62	2.10 ± 0.16*	4.16 ± 0.59	4.25 ± 0.56
MMP-2/TIMP-1, AU/AU	9.35 ± 0.59	3.34 ± 0.56*	2.26 ± 0.35*	0.87 ± 0.39*†
MMP-2/TIMP-2, AU/AU	0.25 ± 0.02	0.25 ± 0.01	0.25 ± 0.01	0.27 ± 0.02
MMP-2/TIMP-3, AU/AU	0.14 ± 0.02	0.18 ± 0.01	0.26 ± 0.02*	0.37 ± 0.04*†
MMP-2/TIMP-4, AU/AU	1.51 ± 0.13	3.98 ± 0.58*	2.68 ± 0.35	2.90 ± 0.90
	(n = 4)		(n = 5)	(n = 8)
MMP-2/TIMP-1, AU/AU	14.88 ± 2.38		2.71 ± 0.84*	0.87 ± 0.39*
MMP-2/TIMP-1, (ng/g)/(ng/g)	2.29 ± 0.49		0.37 ± 0.11*	0.12 ± 0.06*

Table 2. mRNA expression of MMP-2 and TIMPs as well as MMP-2/TIMP ratios in the left ventricle in mice after 3 days of different concentration of norepinephrine (NE; 0.13, 0.25, 0.39 mg/kg·h, s.c., respectively) treatment. Animals with an unfilled mini-osmotic pump replacement for 3 days served as controls (sham). Relative abundance of mRNA expression obtained by RPA detection with 7.5 µg of total RNA was normalized to GAPDH and expressed as arbitrary units (AU). Values are mean ± SEM.; * P < 0.05 vs. sham; † P < 0.05 vs. 0.25 NE; number of measurements in parentheses.

analyzed (Fig. 6A). For this analysis the NE concentration of 0.25 mg/kg per h was chosen. This was the highest NE concentration without increased levels of BNP mRNA expression (Fig. 2C), moderate increase of NE-induced collagen expression (Fig. 4) and without NE-induced increase of LuW/BW ratio (Fig. 3A), all signs of a more pathological phase in the heart remodeling. TIMP-3, which was the most prominent TIMP in sham, was

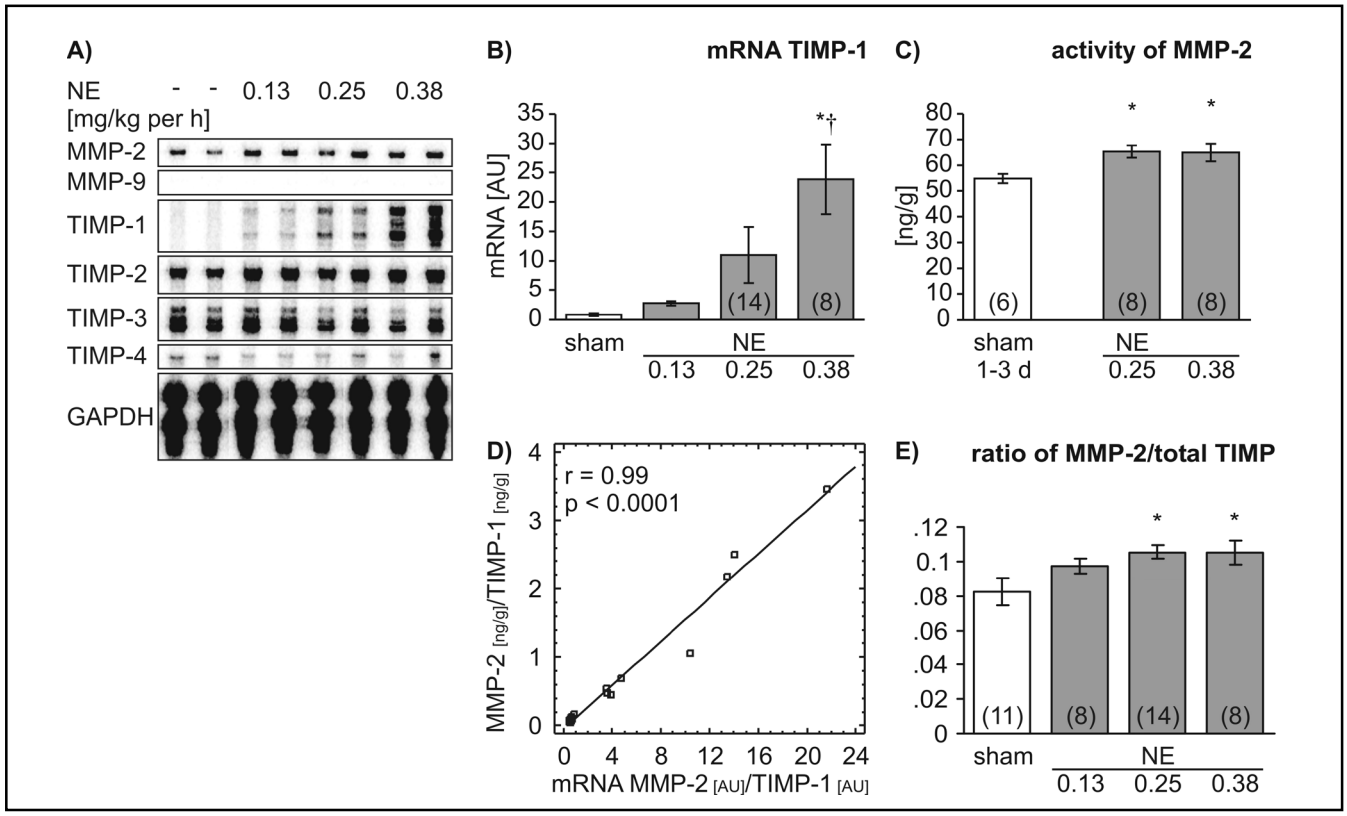

Fig. 5. Changes in the expression of the inhibitor of matrix metalloproteinase (TIMP)-1 and in gelatinolytic activity after treatment with increasing concentrations of norepinephrine (NE) for 3 days in the left ventricle of mice. (A) Representative RPA results of mRNA of MMP-2 and -9, of TIMP-1, -2, -3 and -4 and of GAPDH from 7.5 µg of total RNA from the left ventricle treated 3 days with increasing concentrations of NE (0.13, 0.25 and 0.38 mg/kg per h, respectively) and of sham operated controls (NE -). (B) Quantitative analysis of mRNA expression of TIMP-1 obtained by RPA was related to GAPDH mRNA expression ($x10^{-3}$) and presented as arbitrary units (AU). (C) The MMP-2 activity determined by a specific ELISA is given as the ratio to extracted cardiac protein. (D) The ratio of MMP-2 activity and TIMP-1 concentration was compared with the relative MMP-2/TIMP-1 mRNA ratio. The correlation was analyzed with a linear regression resulted in R-squared value of 97.4%: MMP-2 activity/TIMP-1 = -0.04 + 0.16 *mRNA of MMP-2/TIMP-1 (n = 17). (E) The MMP-2/total TIMP ratio was obtained by the detection of the mRNA by RPA. Values are means ± SEM. *P<0.05 vs. sham; †P<0.05 vs. NE 0.25; number of measurements in parentheses.

elevated only after 4 h. TIMP-1 was increased clearly after 1 day of NE-treatment. It became the most prominent TIMP although it had the lowest expression level in shams in comparison to other TIMPs. This strong elevation was detectable also on the protein level, which was revealed by ELISA of the cardiac tissue extract (Fig. 6B). Protein concentration correlated with the mRNA expression of TIMP-1 (Fig. 6C). This elevated TIMP-1 was also immunohistochemically detectable predominantly in myocytes after 1 day NE treatment (Fig. 6E and G). There were also some TIMP-1-stained myocytes detectable in sham LV (Fig. 6D and F). However, the number of positive myocytes was increased after NE-treatment. TIMP-1 was detectable in vessels both in sham operated controls and after NE-treatment.

Effect of anti-TIMP-1 therapy on cardiac mRNA expression

The effect of the neutralizing AB against TIMP-1 was analyzed after 1 day of NE-treatment, since the expression of TIMP-1 was maximal at this time-point. The NE-induced increase of the mRNA expression of colligin was not changed by administration of the AB (Fig. 7A). The LVW/BW ratio was not changed with NE alone or

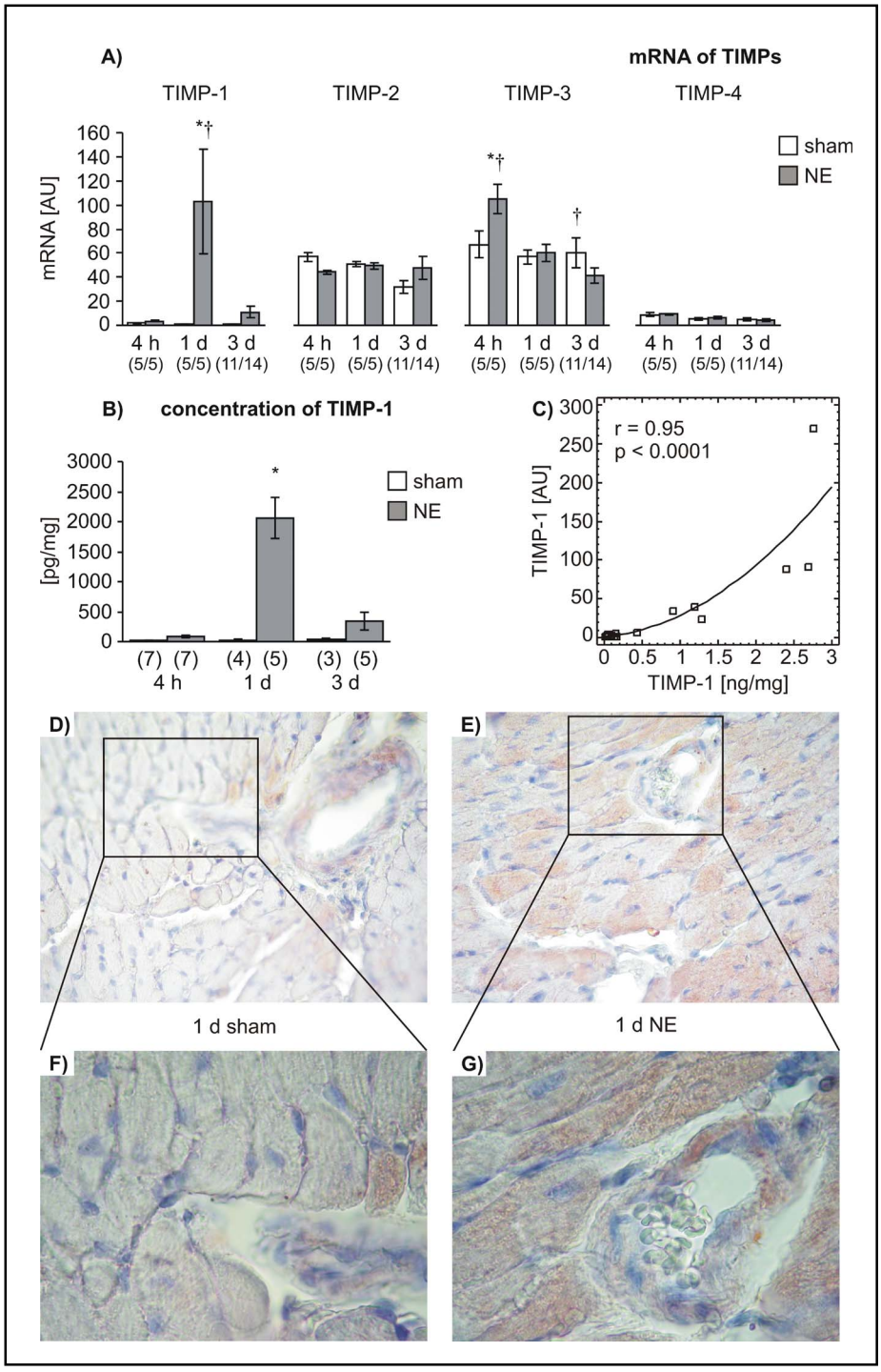

Fig. 6. Time-dependent changes in the expression of the inhibitors of matrix metalloproteinase (TIMP) and localization of the TIMP-1 protein in the left ventricle of mice after norepinephrine (NE)-treatment. (A) TIMP mRNA expression in the left ventricle treated with NE (0.25 mg/kg per h) for different time periods as indicated was compared with sham operated controls. Relative abundance of mRNA expression obtained by RPA detection with 7.5 µg of total RNA was related to GAPDH mRNA expression ($x10^{-3}$) and presented as arbitrary units (AU). (B) TIMP-1 concentration was obtained from ELISA detection of the cardiac tissue. Values are means ± SEM. *P<0.05 vs. time-corresponding sham; †P<0.05 vs. all other TIMPs of same treatment; number of measurements in parentheses. (C) Correlation of TIMP-1 mRNA expression and TIMP-1 concentration was analyzed with the square root-Y model with a R-squared value of 90.7%: TIMP-1 mRNA [AU] = $(1.05 + 0.0041 \cdot \text{TIMP-1 protein [pg/mg]})^2$ (n = 25). (D-G) Representative immunohistochemical staining of TIMP-1 (brown precipitate) after norepinephrine (NE) treatment (E, G) in comparison to sham operated control (D, F) in the left ventricle of mice. Sections of mouse LV with NE-treatment (0.25 mg/kg per h for 1 d) or of sham mice were incubated with anti-mouse TIMP-1 antibody as described in the Method section. After immunostaining the slices were counterstained with haematoxylin (D, E original magnification 72x). F and G (original magnification 180x) display enlarged details of D and E, respectively.

in combination with the TIMP-1 AB (Fig. 7B). The NE-induced elevation of ANP mRNA expression was abolished already with low dose of used neutralizing AB (Fig. 7C). The BNP mRNA expression was not affected with this NE concentration (Fig. 7D).

Discussion

The main finding of this study is that higher doses of NE led to insufficiency of the LV as evidenced by reduced NE-induced elevation of LVSP, contractility and

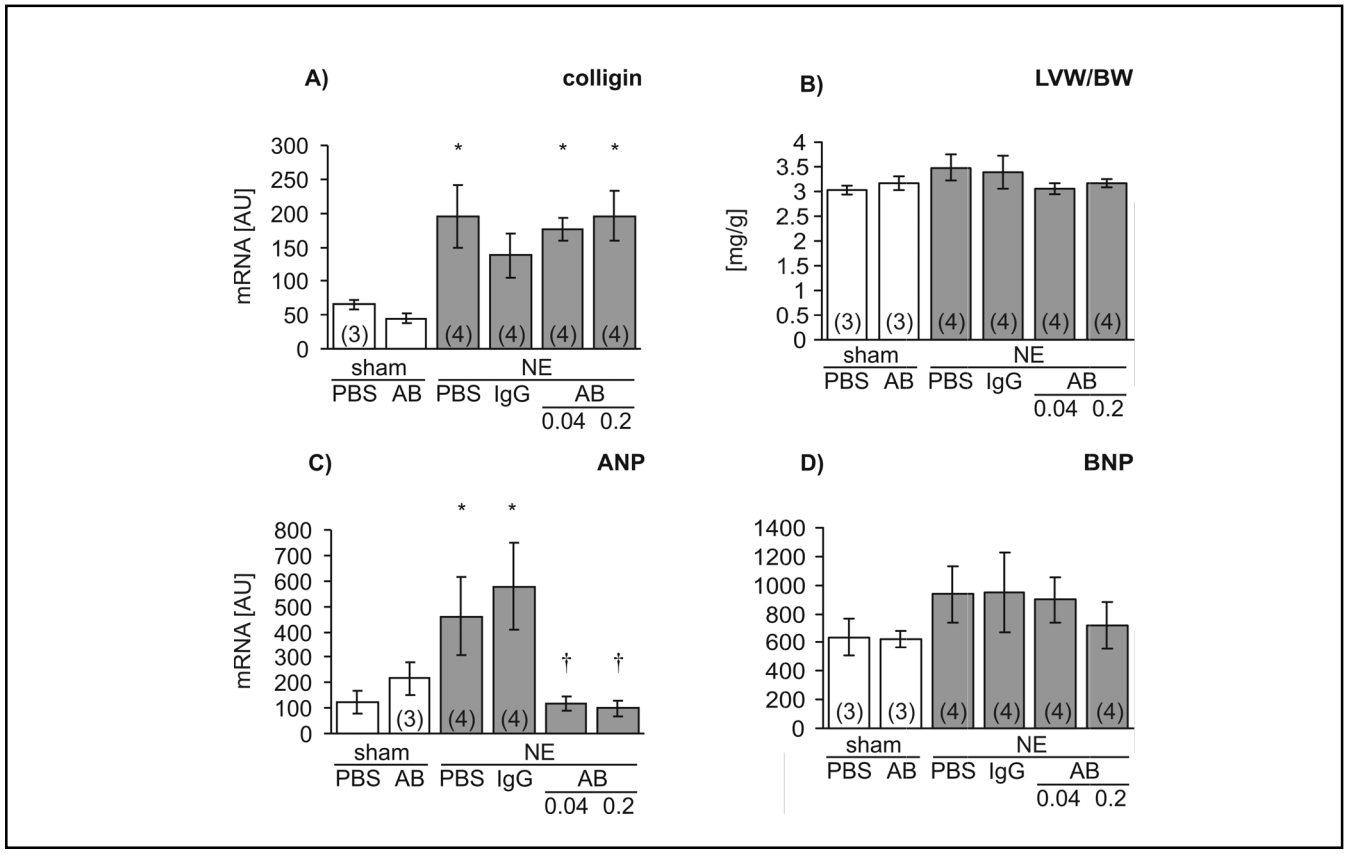

Fig. 7. Effect of neutralizing antibody (AB) against the inhibitor of matrix metalloproteinase (TIMP)-1 on the norepinephrine (NE)-induced elevation of mRNA of colligin (A), the left ventriculatr weight/body weight ratio (LVW/BW, B), the mRNA of atrial (ANP, C) and brain natriuretic peptide (BNP, D) in the left ventricle of 1 day treated mice. Expression of mRNA in the left ventricle was analyzed by RPA detection. The results of NE-treatment (0.25 mg/kg per h) were compared with sham operated controls. Neutralizing goat anti-mouse TIMP-1 AB (sham: 0.2 mg/kg per h, NE: 0.04 or 0.2 mg/kg per h, indicated), 50 µL PBS, or goat IgG control (0.2 mg/kg per h) were injected subcutaneously daily starting one day before NE-treatment. Relative abundance of mRNA expression with 2.5 µg of total RNA was related to GAPDH mRNA expression ($x10^{-3}$) as arbitrary units (AU). Values are means ± SEM. *P<0.05 vs. sham; †P<0.05 vs. NE IgG; number of measurements in parentheses.

relaxation (Tab. 1), and by signs of congestion in the lung (Fig. 3). This was accompanied by evolving LV hypertrophy with elevated collagen, natriuretic peptide and especially TIMP-1 expression. Surprisingly, the NE-induced increase of TIMP-1 expression seems to be more responsible for the induction of the antihypertrophic cardiac factor ANP (Fig. 7C) than for the inhibition of MMP activity (Fig. 5C), which is the classical role of TIMPs.

As described previously in several mammalian species [12-14], treatment with NE induced a concentration dependent increase of LV weight body weight (LVW/BW) ratio (Fig. 2A). Three days of treatment with 0.13 mg/kg per h increased this ratio by 9% in BALB/c mice. The application of a comparable dose for 15 days elevated the LVW/BW ratio by 21% in CD-1 mice [15] and for 18 day by 25% in C57 BL mice [16]. Since elevation of LVW/BW ratio was accompanied by an increase of myocyte diameter in rats after NE treatment [4], it was postulated that NE-induced elevation of LVW/BW ratio in mice also cause an increase of myocyte diameter. Therefore, a NE-induced LV hypertrophy also in mice is postulated. NE-induced cardiac hypertrophy was accompanied by elevation of ANP (Fig. 2B). ANP was already elevated by low dose NE to the same extent as with high dose NE. Therefore, a threshold has to be postulated for the induction of ANP. Thus, above a cer-

tain level of hypertrophy, there is a characteristic level of ANP elevation which cannot be further increased. The 19% elevation of the LVW/BW ratio after 3 days of treatment with 0.38 mg/kg per h NE reflects a very fast and robust hypertrophy reaction. Indeed, the expression of BNP was elevated only with this higher NE-concentration. Since BNP was described as an "emergency" cardiac hormone against ventricular overload [17], this may be a sign for cardiac insufficiency with higher NE doses.

The elevated relative lung weight (Fig. 3A) with hemorrhage (Fig. 3D) observed after treatment with high dose NE was also a sign of LV insufficiency. Furthermore, the attenuation of the NE-induced increase of LVSP, which was seen in rats as well [18], as well as of contractility (dP/dt_{max}) and relaxation (dP/dt_{min}) with higher NE dose supports this notion (Tab. 1). However, the end-diastolic pressure (EDP) was not elevated by NE (Tab. 1). Comparable elevation of lung weight [10], without deterioration in heart function [19], has been observed in an erythrocytosis mouse model in which erythropoietin was overexpressed. NE-induced stress in these mice led to acute heart failure associated with diastolic dysfunction and myocardial ischemia [19]. Therefore, it was postulated that the decrease of NE-induced elevation of heart function together with congestions in the lung was an early sign of heart insufficiency. An additional explanation for the elevated lung weight could be pulmonary venoconstriction with congestion in pulmonary capillaries which has been associated with α-adrenergic stimulation [20]. The NE-induced elevated diastolic aortic pressure (Tab. 1) may reflect the systemic vasoconstriction by the α-adrenergic component of NE stimulation.

The NE-induced development of cardiac hypertrophy in mice was also accompanied by sustained activation of ECM metabolism similar to the situation of NE-induced hypertrophy in rats [4]. The expression of collagen type I and type III was clearly more elevated with high dose NE (Fig. 4B and C, respectively). This is an additional sign for a more pathological stage in heart remodeling. The ratio of type III/type I collagen was increased concentration dependently by NE only up to the concentration of 0.25 mg/kg per h (Abb. 4D). The shift to collagen type III, which is responsible for a more cross-linked collagen network, could be a sign for the adaptive phase of LV remodeling. The lack of further increase of this ratio at high dose NE could also be a sign for a change to pathological remodeling. The stiffer ventricle is characterized by a predominance of collagen type I with its fibril forming quality which is the result of pathological remodeling of ECM [21].

In contrast to the NE-induced remodeling in rats [7, 19], the elevation of MMP-2 activity in mice (Fig. 5C) was not accompanied by increased mRNA expression of MMP-2 (Tab. 2). However, the elevation of the MMP-2 activity could be explained by the decrease of total TIMP concentration reflected by the increase of MMP-2/total TIMP ratio based on mRNA detection (Fig. 5E). The measurement of all TIMPs is necessary to predict the activity of MMP-2 since calculation of the ratio of MMP-2 to single TIMPs showed no significant difference in the case of TIMP-2, a concentration dependent increase in the case of TIMP-3 or was even decreased with TIMP-1 (Tab. 2). The strong concentration dependent increase of TIMP-1 mRNA (Fig. 5B) and the concentration dependent decrease of the MMP-2/TIMP-1 ratio implicated a different function of TIMP-1 than inhibiting the activity of MMP-2. Which function has the NE-induced elevation of TIMP-1?

Although the canonical role that has been ascribed to TIMPs in the heart is that of neutralizing active MMPs, there is increasing evidence that TIMPs may exert "non-traditional" effects that are independent of their ability to inhibit MMPs [22]. TIMP-1 was first identified to stimulate erythropoiesis [23] and subsequently as an agent that stimulates the growth of cell lines [24]. The importance of TIMP-1 in the NE-induced early remodeling of the heart was more obvious after the analysis of the kinetic of NE-induced TIMP mRNA expression (Fig. 6A). TIMP-1 became the most prominent TIMP after 1 day of NE-treatment, which correlated with NE-induced elevation of the protein level of TIMP-1 (Fig. 6C) predominantly in myocytes (Fig. 6E and G).

One of the "nontraditional" effects of TIMPs is the stimulation of collagen expression which was shown for TIMP-2 by overexpressing of this TIMP in cardiac fibroblasts [25]. The function of TIMP-1 was not the induction of elevated collagen production, since the NE-induced increase of the expression of colligin was not suppressed by the neutralizing TIMP-1 AB (Fig. 7A). NE-induced elevation of collagen expression was not affected by TIMP-2, since its expression was not changed by NE (Fig. 5A). The NE-induced elevation of TIMP-1 seems to be necessary for the NE-induced elevation of ANP expression, since the NE-induced increase of ANP was suppressed by the neutralizing TIMP-1 AB (Fig. 7C). The elevation of ventricular ANP expression is traditionally interpreted as part of the fetal gene program, which is induced during cardiac hypertrophy. Now, there are a lot of evidence indicating that ANP is a local antihypertrophic cardiac factor [26]. Its induction during

NE treatment may prevent an excessive cardiac hypertrophy. This ANP-induced attenuation of NE-induced hypertrophy may be mediated by TIMP-1. However, the mechanism of the postulated TIMP-1 induced ANP stimulation is still unknown.

We provide evidence that during NE-induced development of LV hypertrophy, induction of collagen type I and type III expression was accompanied by concentration and time dependent elevated of TIMP-1 expression in mice. TIMP-1 had the highest concentration among the TIMPs after 1 day of NE-treatment, although only 1% was found in sham operated controls. The elevated expression of TIMP-1 by NE predominantly observed in myocytes may induce the elevation of the antihypertrophic cardiac factor ANP, since NE-induced elevation of ANP expression was abolished by neutralizing TIMP-1 AB. Thus, TIMP-1 may mediate ANP-induced retardation of NE-induced hypertrophy. Higher concentration of NE induced insufficiency of the LV indicated by lung congestions and attenution of NE-induced increase of heart function.

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